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APPLICATION NUMBER: *60/443,225*
FILING DATE: *January 27, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/02409*

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COMMISSIONER OF PATENTS AND TRADEMARKS

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR §1.53(c).

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
William G.	Tong	San Diego, CA

[] Additional inventors are being named on the 0 separately numbered sheets attached hereto.

TITLE OF THE INVENTION (280 characters max)

Sensitive Sensing Based on Optical Nonlinear Wave Mixing

CORRESPONDENCE ADDRESS

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[X] Customer Number: 20985

OR

[ ] Firm *or* Individual Name					
Address					
Country	United States	Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages* 49 [] CD(S), Number

[] Drawing(s) *Number of Sheets* [] Other (specify)

[] Application Data Sheet. See 37 CFR 1.76.

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X] Applicant Claims small entity status. See 37 CFR 1.27.

[X] A check or money order is enclosed to cover the filing fees.

[X] The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 06-1050

FILING FEE AMOUNT ($): $80

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Signature

Name Bing Ai, Reg. No. 43,312

Date January 27, 2003

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SENSITIVE SENSING BASED ON OPTICAL NONLINEAR WAVE MIXING

[0001] This application relates to optical sensing of various materials, including chemical and biological substances. Optical sensing devices and techniques of this application are designed as a highly sensitive, selective and high-resolution sensors based on multi-photon nonlinear laser wave mixing and may be implemented using, among others, microfluidic devices, laboratory-on-a-chip, fiber optics, and capillary cells for potential chemical, biological and environmental applications.

[0002] Nonlinear optical wave mixing may be implemented in optical sensing systems with different configurations. Examples of such configurations may be found in U.S. Patent Nos. 5,600,444 issued Feb. 4, 1997 and 6,141,094 issued Oct. 31, 2000, which are attached here as part of this application. Techniques and features in the above-referenced patents may be used or combined with the techniques described in this application. Notably, the nonlinear multi-photon laser wave-mixing optical methods may be implemented in portable, robust and compact systems for a wide range of applications, including, but not limited to, biomedical applications. Advantages of such techniques and systems include high spatial resolution and sensitivity, versatile applications, ease of operations, convenient procedures, and less tedious chemical steps, etc. Taking advantage of small probe volumes available, this method

can be easily interfaced and adapted to small, relatively portable, robust, microfluidic devices.

[0003] As an example, the nonlinear wave mixing techniques for sensitive high-resolution detection may be implemented with high temperature atomizers including graphite discharge plasmas, graphite furnace, inductively coupled plasma, and flame atomizers with detection sensitivity levels in the sub-parts-per-quadrillion levels (see my 2000 patent). Applications to liquid-phase samples can achieve high detection sensitivity levels.

[0004] These new methods offer many potential applications in many fields including chemistry, biology, and medicine. For example in biotechnology, these could be used for detecting biomolecules (e.g., proteins, DNAs, etc.) with or without labels or tags, for studying enzyme activities, for monitoring smaller chemical/biological changes more dramatically with less tedious procedures, for studying bio molecular structures, for analysis of small bio cells with high spatial resolution, for sensitive detection as sensors, and many other potential applications.

[0005] Exemplary features of the present wave-mixing based sensing techniques are listed below.

[0006] 1. The present laser-based detection method may be useful for various applications in a wide range of fields for measuring atoms, isotopes (gas-phase) and molecules (liquid-

phase) at detection levels currently not available (i.e., 1,000 to 1,000,000 times better detection sensitivity).

[0007] 2. Preliminary detection limits may be obtained at sub-parts-per-quadrillion level, sub-attogram, sub-zeptomole, and sub-femto molar detection limits.

[0008] 3. The present technique can be effective when interfaced to popular gas-phase atomizers and liquid-phase flow systems.

[0009] 4. Sensitive detection may be realized in gas-phase atoms and isotopes at sub-Doppler spectral resolution and sensitive detection of liquid samples.

[0010] 5. Generation of a coherent (i.e., laser-like) analytical signal beam is used to enhance signal-to-noise ratio.

[0011] 6. Absolute positive signals can be measured against dark background.

[0012] 7. Nanoliter- and picoliter-level probe volumes and spatial resolution can be achieved.

[0013] 8. Efficient use of short absorption path lengths.

[0014] 9. Simple two-input-beam planar (2-D) and three-input-beam non-planar (3-D) optical configurations.

[0015] 10. Use of aperture templates for very effective and reliable optical alignment.

[0016] 11. Inherently easy interface for other chemical instruments including, but not limited to, gas chromatographs

(GC), liquid chromatographs (LC), mass spectrometers (MS), GC-MS, LC-MS, inductively coupled plasmas (ICP), ICP-MS, high performance/power capillary electrophoresis (HPCE) systems, flow injection analysis (FIA) systems and other similar chemical separation and analytical techniques.

[0017] 12. Applicable to fluorescing, weakly fluorescing, and non-fluorescing samples.

[0018] 13. Low laser power requirements (milliwatts for CW lasers, and nanojoules for pulsed lasers)

[0019] 14. Effective use of small, low-power, compact, inexpensive solid-state lasers.

[0020] 15. Stable optical alignments (i.e., signal "on" without major realignment).

[0021] 16. Effective use of polarization, wavelength and other modulation methods.

[0022] 17. Phase conjugate signal properties for unique applications.

[0023] The new methods offer many potential applications in chemical, biological and environmental fields and many advantages over other methods (e.g., fluorescence) including, but not limited to, applicability of both fluorophores and chromophores, better detection sensitivity, better monitoring of small changes in chemical and biological properties (i.e.,

quadratic signal dependence on concentration), and easier signal collection (virtually 100% collection efficiency).

[0024] The present techniques may be applied in the following areas: application of laser wave-mixing spectroscopy for thin-film sensors; application of laser wave-mixing spectroscopy for fiber optics; application of laser wave-mixing spectroscopy for laboratory-on-a-chip; application of laser wave-mixing spectroscopy for capillary cells, sensitive detection of biomolecules with or without labels and tags, sensitive detection of analytes in picomolar, femtomolar and lower concentration levels; sensitive detection of analytes at nanoliter and picoliter-level probe volumes and spatial resolutions (the overlapping volume for the input beams is very small); sensitive detection of both chromophores and fluorophores; applicable to fluorescing, weakly fluorescing and non-fluorescing samples, and hence, yielding excellent fluorescence-like detection limits for samples that do not fluoresce; multi-photon laser wave-mixing optical arrangements based on 3-beam, one-laser, simple optical configurations for gas-phase samples; multi-photon laser wave-mixing optical arrangements based on 2-beam, one-laser, simple optical configurations for liquid-phase samples; template-based optical alignment allows the use of two specially designed templates for effective and reliable forward-scattering optical alignment;

inherently easy interface of these sensitive laser detection methods to many popular continuously flowing liquid-phase chemical instruments including, but not limited to, high performance liquid chromatographs, high performance/power capillary electrophoresis systems, flow injection analysis systems and other similar chemical separation and analytical techniques; inherently easy interface of these sensitive laser detection methods to other gas-phase chemical instruments including, but not limited to, mass spectrometers, gas chromatograph-mass spectrometers (GC-MS), liquid chromatograph-mass spectrometers (LC-MS), inductively coupled plasma-mass spectrometers (ICP-MS), and other similar analytical methods.

[0025] Unique features of this laser method allow the effective use of lasers as compared to the conventional light bulbs currently used in commercially available analytical instruments. Detection sensitivity levels may reach orders of magnitude better than currently available models (1,000 to 1,000,000 times, depending on chemical properties, experimental parameters, and optoelectronics).

The following sections describe several implementation examples to illustrate the above features and advantages.

IMAGING OF DNA MICROARRAYS BY LASER WAVE MIXING

[0026] Two-dimensional imaging of a DNA microarray is accomplished rapidly using laser wave mixing with the use of an argon ion laser operating at 515 nm. Intra-spot spatial resolution is obtained with high reproducibility. Imaging of a single row of 10 spots is also performed with high reproducibility. Laser wave-mixing signal is confirmed by monitoring a nonlinear dependence of signal on laser power (i.e., a slope of 2.85 is measured as compared to the expected theoretical slope of 3). The lowest concentration of probe detected that is hybridized with the DNA-microarray is 1 femto molar (fM) (i.e., 0.8 attogram per microliter or 0.1 molecule per square micrometer). Research is in progress and more work is under way to further improve these detection sensitivity levels.

[0027] Processing procedure (routine steps previously reported) may be implemented as follows. Suitable DNA microarrays such those marketed by TeleChem may be used. Oligonucleotides are attached to a superamine substrate surface, which contains primary amine groups on a glass slide that carry a positive charge at neutral pH. This charge permits the formation of ionic bonds with the negatively charged phosphate backbone of the DNA. A covalent bond is then formed between the DNA and the surface by ultraviolet light or heat treatment. Chip microarrays are printed with 70-mer oligonucleotides in 200

μm spots and configured as two identical 10 x 10 sub grids that are spaced 4.5 mm apart. Each microarray chip purchased is further processed to remove unbound target sequences prior to hybridization. The chip is subjected to a 2 minute wash at 25 °C in 2 x SSC + 0.1% sarkosyl solution. This step creates an ionic atmosphere on the chip so that the printed oligos do not aggregate together. The chip is then washed for 2 minutes in 2 x SSC solution. This step removes sarkosyl from the array. Sarkosyl is a detergent that can disrupt future binding reactions. The microarrays are then treated for 2 minutes at 100 °C in D.I. H2O and cooled to room temperature. The boiling water denatures the oligos and helps assure that the oligos are straight for hybridization. Finally, the microarrays are subjected for 2 minutes in ice-cold 100% ethanol.

[0028] Hybridization procedure (routine steps previously reported) may be implemented as follows. Microarray chips are hybridized in a hybridization cassette for approximately 4 hours at room temperature. Hybridization takes place under a 22 mm x 22 mm optically flat cover slip with a 5.0 μL probe solution. The probe solution contains 8.0 μL of 1.25 x SuperHyb hybridization buffer and 2.0 μL of the universal probe solution. The universal probe solutions contain the complimentary 9-mer oligonucleotide strands. The 9-mer strands have a Cy3 label on the 5′ end. The microarrays are then washed for approximately 1

minute in buffer solutions A, B, and C, which contain 2 x SSC + 0.1% sarkosyl, 2 x SSC and 0.5 x SSC, respectively. These final washing steps break bonds that are formed from the non-specific matching of the probe. The slides are then whipped dried for 2 minutes before 2-dimensional imaging by laser wave mixing.

MICROARRAY IMAGING BY LASER WAVE MIXING

[0029] Microarrays are probed, excited, scanned and measured by using a novel forward-scattering degenerate four-wave mixing optical setup based on the absorption of the Cy3 label, which has an absorbance maximum near 535 nm. The 514.5 nm line of an argon ion laser is used as the excitation source for the Cy3 label. The resulting signal is sent through a custom designed precision template and detected by a photodiode. During the spatial scanning process, the laser power used is between 2.5 and 5 mW, but not limited to this range. Microarray spots are scanned with the use of a motorized precision actuator in order to achieve automated and reproducible scans. Background optical noise is determined by scanning the blank glass surface between the spots (i.e., the optical blank). No signal is detected on the blank glass surface, assuring that hybridization and washing are performed effectively. Detection sensitivity is excellent and improving continuously. Our preliminary results indicate that laser wave mixing is one of the most sensitive detection

techniques for microarray applications. Laser wave mixing also allows intra-spot spatial resolution where one can scan, probe and measure bio/chemical contents "within" a single spot on the microarray. Preliminary intra-spot scanning and probing show inhomogeniety within each spot due to inhomogeniety in manufactured preprinted spots. Our laser probe diameter is 25 μm, and hence, it requires much smaller amount of reagents for detection. The microarray chips we purchased come with a marker spot. The marker spots located in the corners of each sub-grid contain Cy3-labeled control oligonucleotides. We can even distinguish variations in signal intensity that are due to differences in sequence composition of the 70-mer oligonucleotide targets.

MICROARRAY DETECTION SENSITIVITY BY LASER WAVE MIXING

[0030] Our preliminary limit of detection (LOD) so far for the hybridized probe detected is 1 fM (femto molar or 1 x 10-15 molar). This is better than those published so far by any research group. Reproducible results are also obtained when scanning single-spot profiles repeatedly and when scanning single-row profiles repeatedly. Laser wave-mixing signal is also confirmed by monitoring a nonlinear dependence of signal on laser power (i.e., a slope of 2.85 was measured as compared to the expected theoretical slope of 3)

SEPARATION AND DETECTION OF Ni AND Cu BY MICROCHIP-BASED CAPILLARY ELECTROPHORESIS USING LASER WAVE MIXING

[0031] Laser wave mixing, an unusually sensitive optical absorption based method, is used to detect Cu and Ni (the technique is not limited to these metals) after they are separated by a capillary electrophoresis (CE) system embedded on a microchip. The metal ions are chelated with 4-(2-Pyridylazo) resorcinol (PAR) prior to separation. Wavelength shift by PAR allows optical detection using the 514.5 nm line of an argon ion laser. The lowest concentration detected are 0.26 parts-per-billion and 16 parts-per-billion for Ni and Cu, respectively. This corresponds to excellent preliminary detection limits (S/N = 2) of 75 parts-per-trillion and 864 parts-per-trillion for Ni and Cu, respectively, using this compact microchip-based chemical separation/analysis system.

MICROCHIP-BASED CAPILLARY ELECTROPHORESIS CHEMICAL SEPARATION SYSTEM WITH LASER WAVE-MIXING DETECTION

[0032] A forward-scattering degenerate four-wave mixing optical setup is interfaced to a microchip with built-in microchannels for capillary electrophoresis separation and detection. The 514.5 nm line of an argon ion laser is used as the excitation source with a laser power of 50 mW. A photodiode is used to monitor the signal beam resulting from the

microchannel on the chip. Four platinum wires inserted in the access holes of the microchip serve as electrodes for the capillary electrophoresis system. The CE-microchip, obtained from Micralyne, Inc. (Alberta, Canada) is made of Borofloat glass. The microchip substrate is 95 mm long, 16 mm wide and 2.2 mm thick. The microchip (Twin T type) consists of an offset microchannel cross with 8 mm long side arms for sample injection, and an 85 mm long separation and detection channel. The channels are 50 μm wide and 20 μm deep. A 30 kV power supply (Model PS/MJ30P0400-11, Glassman, New Jersey) is used to power the microchip-based CE system via platinum electrodes. The PAR agent is used to chelate the metal ions with +2 charge at a concentration of 1 x 10-3 M in a 10 mM ammonium phosphate buffer at pH 7.5. The metal ion stock solution is obtained from Fisher. A Millipore water system provides the water for sample preparation and sample solutions are filtered with a 0.22 μm syringe filter. The final low-concentration metal solutions for detection sensitivity studies are obtained by serial dilution from a stock solution.

[0033] The PAR buffer solution is filtered before injection into the microchip. A series of low-concentration metal sample solutions are prepared by serial dilutions. The microchip is first conditioned with the PAR-buffer solution and a baseline laser wave-mixing signal is obtained based on the slight optical

absorption at 514 nm. Once the wave-mixing alignment is optimized based on this baseline signal, a metal sample solution prepared in the same PAR-buffer solution is injected using an 8 kV injection voltage. The power supply is then switched from the injection mode to the separation mode, using an 8 kV separation power. The PAR-metal complexes are separated as they travel down the separation channel on the microchip since the pKa of the p-phenolic group of PAR is influenced differently by the central metal ion. Copper elutes first after 20 s. and then Ni at 80 s. Hence, chemical separation of this metal pair is very effective with total resolution. Other metals can be separated with excellent resolution using similar parameters, or if necessary, CE voltage levels can be adjusted conveniently to enhance separation resolution. Rinsing and conditioning of the microchip are done using a syringe pump connected to the microchip via a plastic tubing. The plastic tubing is interfaced to the microchip at the microchip access hole via a pipette tip. Rinsing and conditioning are performed before each sample run to flush out any residue and to avoid any contamination. The capillary electrophoresis system used in this setup is a home-built custom-designed system with manual electrode switching between the injection mode and the separation mode with the high-voltage power turned off.

MICROCHIP-BASED LASER WAVE-MIXING DETECTION SENSITIVITY

[0034] For detection sensitivity studies, a 0.26 parts-per-billion (ppb) Ni sample solution is injected using a 57 s. injection time and a 8 kV injection voltage. This particular design of the microchip does not allow a wide range of sample size injected. One must allow sufficient injection time in order to assure that the sample plug is injected into the side-arm injection microchannel. The injection time (about 50 s.) for this microchip-based capillary electrophoresis system is slightly longer than that for a conventional column-based capillary electrophoresis system. The sample mixture is separated under 8 kV of capillary electrophoresis separation power and detected by laser wave mixing 85 s. later. A preliminary detection limit (S/N = 2) of 75 parts-per-trillion (ppt) is determined for Ni using this microchip-based laser wave-mixing system. For Cu detection sensitivity studies, a 16 ppb Cu sample solution is injected using a 70 s. injection time. The Cu peak is detected 53 s. later using an 8 kV separation power. A preliminary detection limit (S/N = 2) of 864 parts-per-trillion (ppt) is determined for Cu. Since Ni has a higher absorption at 515 nm as compared to Cu, Ni yields better laser wave-mixing detection sensitivity. Our detection sensitivity level for Ni on this microchip is better than those published so far by any research group for Ni on microchips.

[0035] Only a few implementations are disclosed. However, it is understood that variations and enhancements may be made without departing from the spirit of and are intended to be encompassed by the following claims.

Claims

What is claimed is the devices and techniques as described and illustrated.

US006141094A

United States Patent [19]

Tong

[11] Patent Number: 6,141,094

[45] Date of Patent: *Oct. 31, 2000

[54] SENSITIVE LASER SPECTROSCOPIC DETECTION BASED ON THREE-DIMENSIONAL NONLINEAR FOUR-WAVE MIXING

[75] Inventor: William G. Tong, San Diego, Calif.

[73] Assignee: San Diego State University Foundation, San Diego, Calif.

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: 09/399,930

[22] Filed: Sep. 21, 1999

Related U.S. Application Data

[60] Provisional application No. 60/116,524, Jan. 20, 1999.

[51] Int. Cl.[7] G01J 3/02; G01N 21/74

[52] U.S. Cl. 356/300; 356/312

[58] Field of Search 356/300, 326, 356/328, 312

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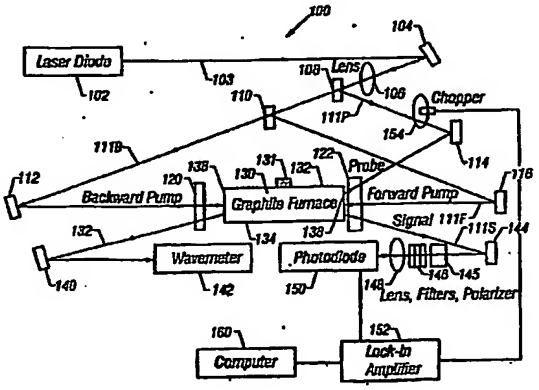
Primary Examiner—F. L. Evans

Attorney, Agent, or Firm—Fish & Richardson P.C.

[57] ABSTRACT

Devices and techniques for performing highly-sensitive spectroscopic measurements in a sample vapor by using a four-wave-mixing optical system and an atomizer chamber. One embodiment of a spectrometer comprises a gas-phase atomizer having an atomizer chamber operable to vaporize a sample solution to produce a sample vapor, first and second alignment templates having apertures to align a probe beam, first and second pump beams to form a four-wave mixing configuration, a laser tunable to generate a laser beam at a desired wavelength corresponding to an absorption line in the sample vapor, and a set of optical elements disposed relative to the laser and the atomizer to split the laser beam into the probe beam, the first pump beam, and the second pump beam. The probe beam, the first and second pump beams are directed to overlap with one another in the sample vapor to produce a signal beam through a four-wave mixing process.

25 Claims, 5 Drawing Sheets



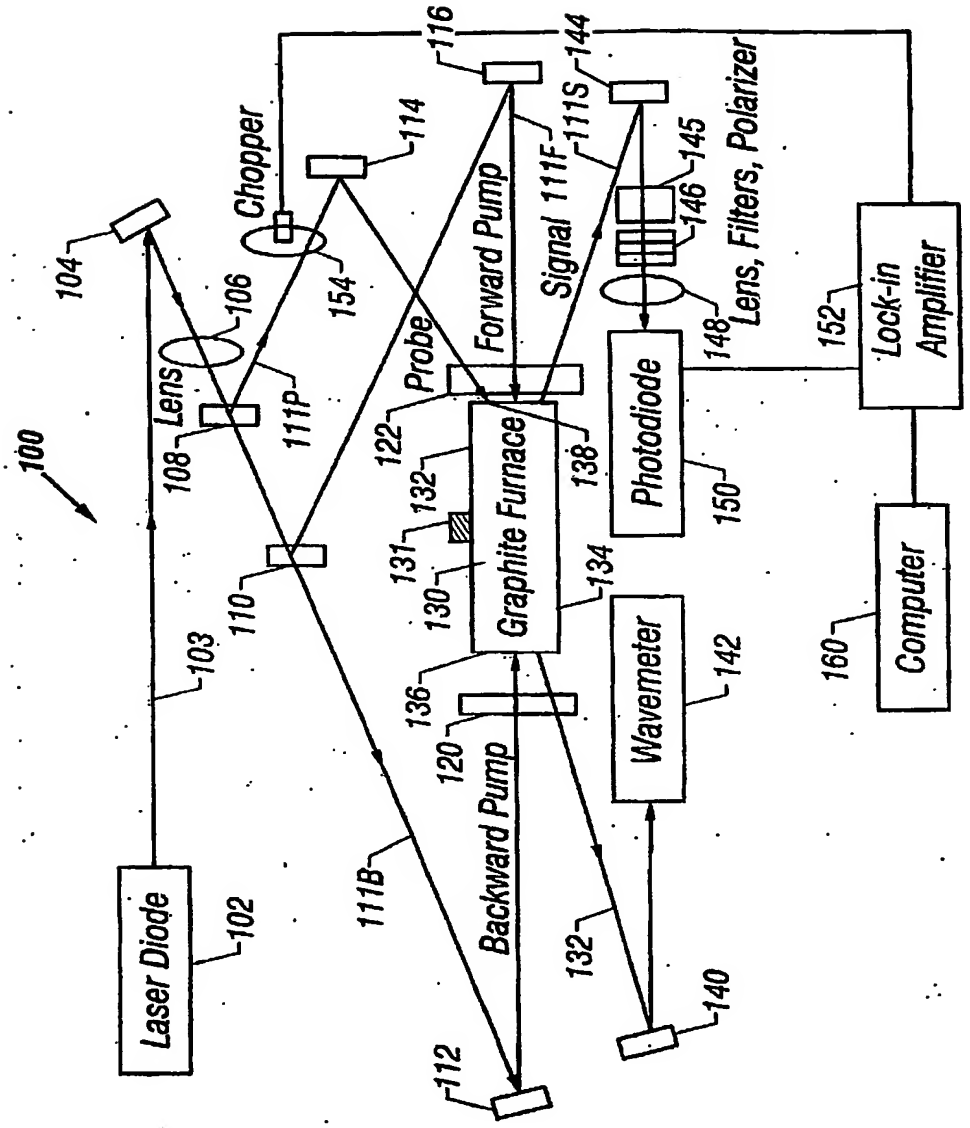

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FIG. 1

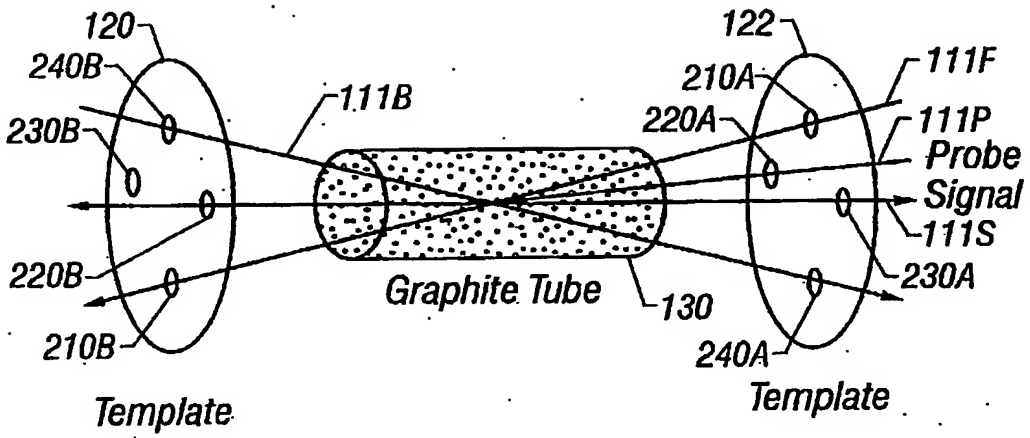

FIG. 2

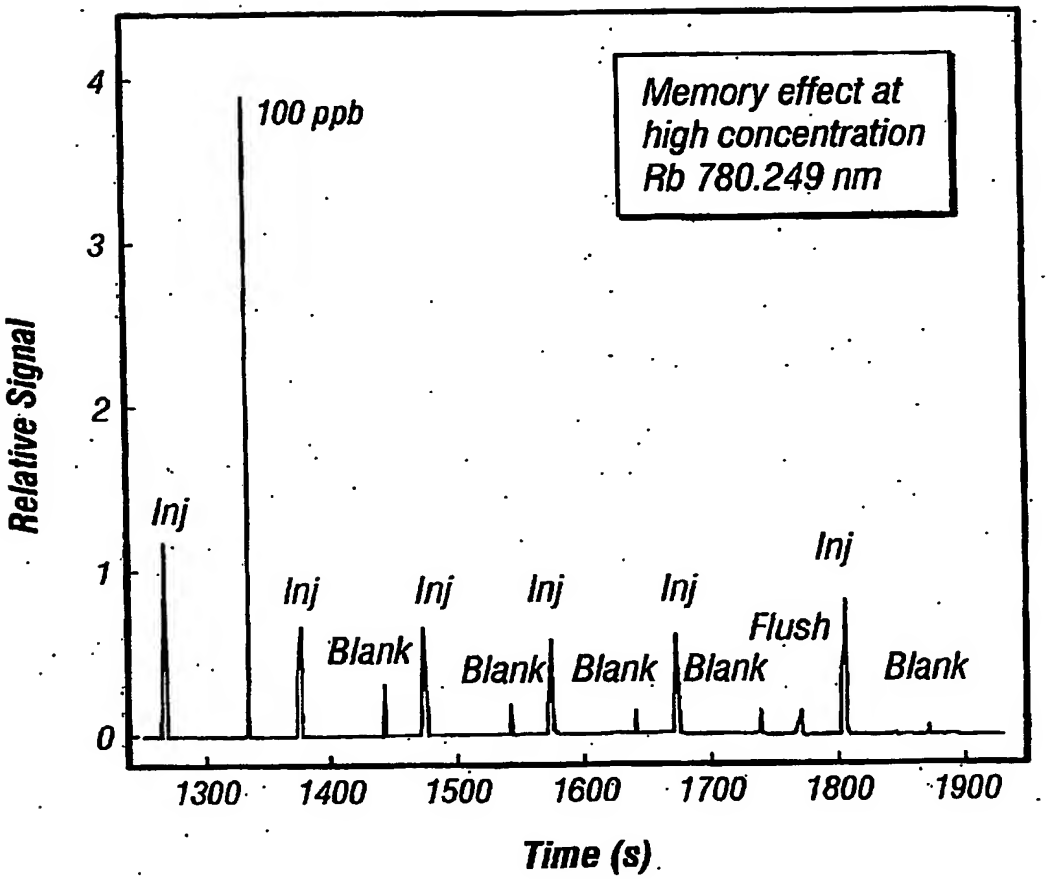

FIG. 3

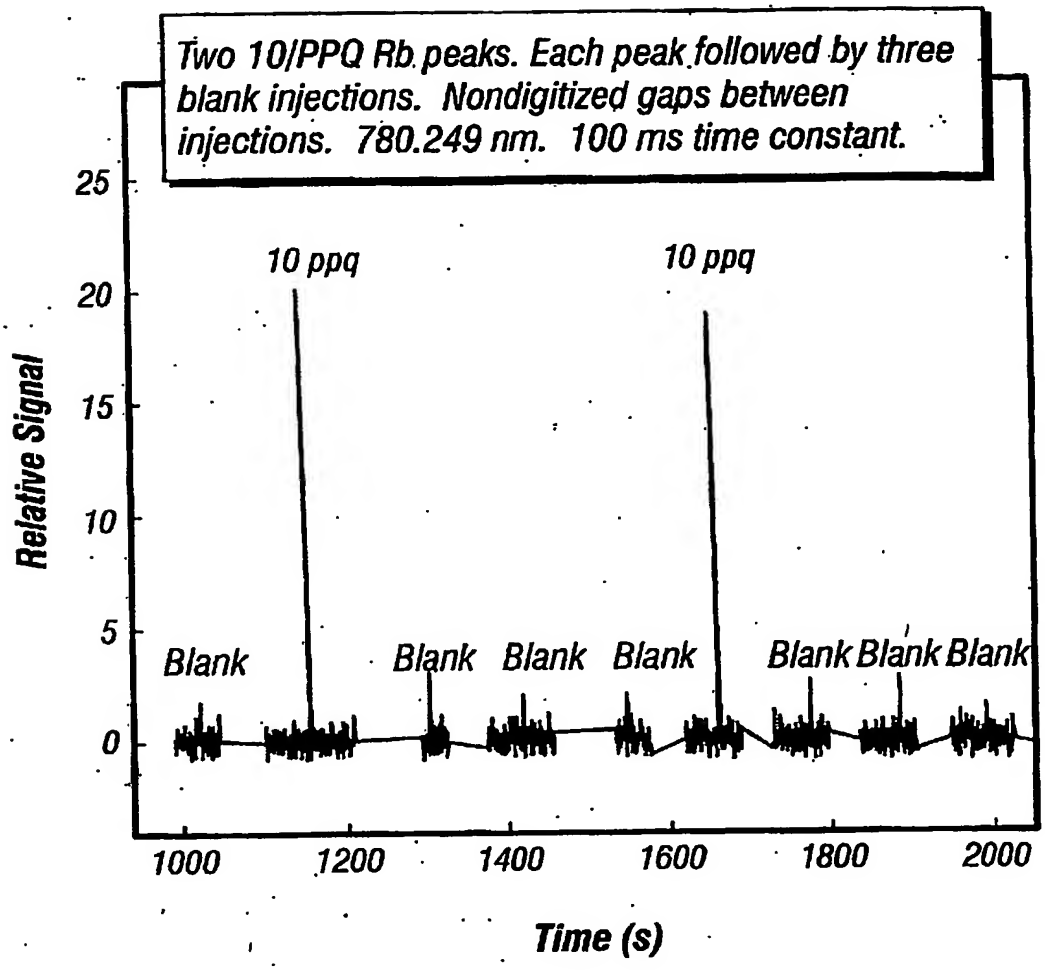

FIG. 4

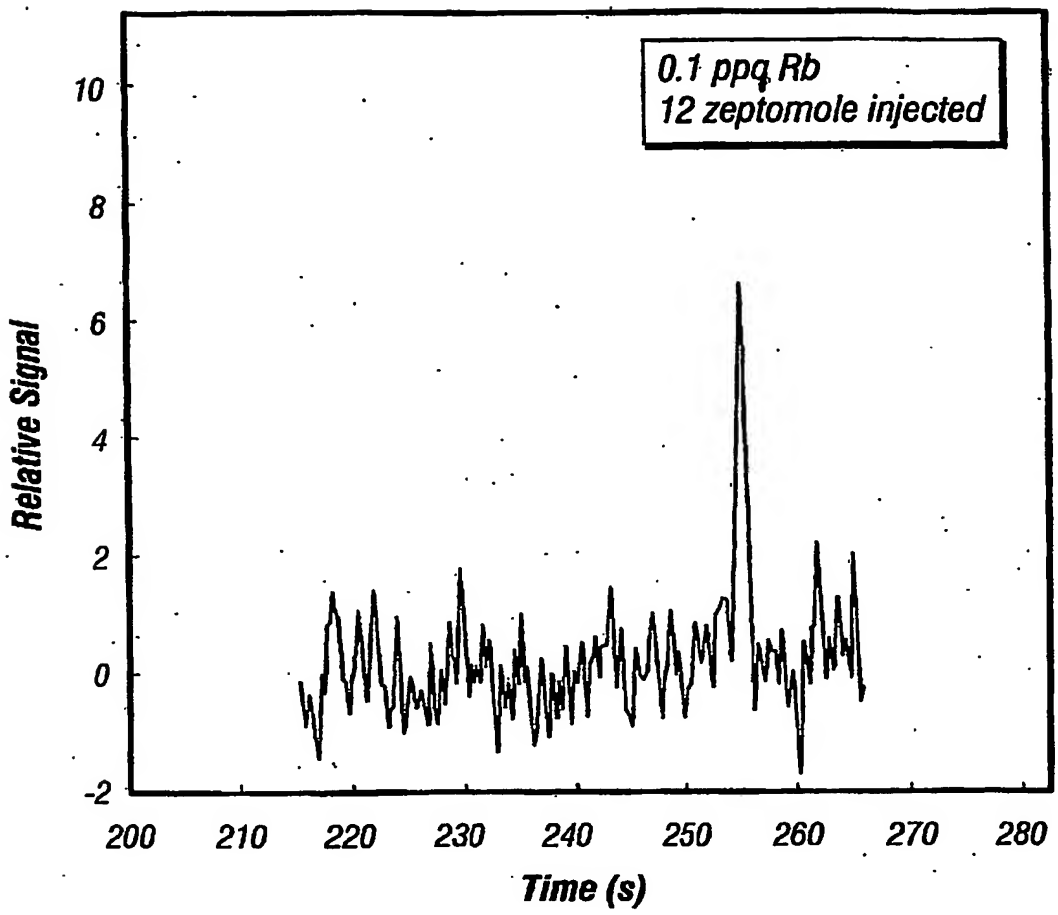

FIG. 5

SENSITIVE LASER SPECTROSCOPIC DETECTION BASED ON THREE-DIMENSIONAL NONLINEAR FOUR-WAVE MIXING

CLAIM OF PRIORITY

This application claims priority under 35 U.S.C. §119(e) to U.S. patent application Ser. No. 60/116,524, filed Jan. 20, 1999.

TECHNICAL FIELD

This application relates to detection of minute substances, and more specifically, to spectroscopic detection techniques based on nonlinear four-wave mixing in optical media.

BACKGROUND

Four-wave mixing is a process in an optical medium where three coherent optical waves interact with one another through nonlinear coupling to produce a fourth coherent signal wave. The third-order nonlinear susceptibility of the medium primarily contributes to such nonlinear coupling. The signal wave includes information on optically-excited atoms or molecules present in the medium where the three input optical waves intersect and hence can be collected to extract information about the medium. For example, the signal strength of the signal wave is associated with the population of atoms or molecules and the spectral characteristics of the signal wave can be analyzed to reveal the structure of the atoms or molecules of interest. The coherent characteristics of the four-wave mixing signal beam have a number of advantages, including a laser-like signal beam, efficient signal collection, excellent spatial resolution, and sub-Doppler spectral resolution. Hence, four-wave mixing has been widely used as a highly sensitive tool in spectroscopic measurements and many other applications requiring detection of a minute amount of a substance.

One commonly-used four-wave mixing process is the backward-scattering degenerate four-wave mixing where three input beams (two pump beams and one probe beam) are at a common frequency. The nonlinear wave mixing produces a fourth signal beam at the same common frequency as the input beams. In a two-dimensional configuration, the wave vectors of the three input beams are in the same plane when mixed inside the medium. Due to conservation of momentum, the wave vector of the generated signal beam is also in the same plane. When two pump beams counter propagate and the probe beam intersects the pump beams at a small angle (e.g., less than 1 degree), the resulting signal beam is a time-reversed replica of the probe beam and propagates in the opposite direction of the probe beam. A beam splitter can be used to separate the signal beam from the path of the probe beam for signal detection. In a three-dimensional configuration, the pump beams may be in the same plane and the probe beam may be in a different plane. Hence, the generated signal beam will not retrace the probe beam and can be directly collected by a photodetector without using a beam splitter. This increases the signal strength of the received signal and improves the signal-to-noise ratio.

The above four-wave mixing detection can be used to form a spectroscopic analyzer by coupling a four-wave mixing optical module to an atomizer. This spectroscopic analyzer can be used to analyze gas-phase, liquid-phase, and solid-phase samples. The atomizer vaporizes an analyte to produce a vapor sample and the four-wave mixing optical module performs optical measurements of the vapor sample. Such a spectroscopic analyzer may be used in a range of applications, including trace-concentration analysis using gas-phase atomizers with sample cells, circular dichroism spectroscopy, capillary electrophoresis, and liquid chromatography in various fields such as biotechnology, environmental, material engineering and science, and basic scientific research.

SUMMARY

The invention includes an optical spectrometer based on optical nonlinear four-wave mixing to achieve a high detection sensitivity of measuring minute substances with a concentration on the order of one part per 10^{15} or less. One embodiment of the spectrometer comprises a gas-phase atomizer having an atomizer chamber operable to vaporize a sample solution to produce a sample vapor, first and second alignment templates having apertures to align a probe beam, first and second pump beams to form a four-wave mixing configuration, a laser tunable to generate a laser beam at a desired wavelength corresponding to an absorption line in the sample vapor, and a set of optical elements disposed relative to the laser and the atomizer to split the laser beam into the probe beam, the first pump beam, and the second pump beam. The probe beam, the first and second pump beams are directed to overlap with one another in the sample vapor to produce a signal beam through a four-wave mixing process.

The gas-phase atomizer includes an atomizer chamber that has a first optical window to receive the probe beam and the first pump beam, and a second optical window opposing the first optical window to receive the second pump beam. The atomizer chamber may include a top wall and a bottom wall where the sample solution is injected and vaporized towards the top wall. The apertures of first and second alignment templates may be so arranged and positioned that the probe beam, the first and second pump beams overlap in a region is closer to the top wall than the bottom wall so as to reduce light scattering.

The first and second alignment templates may be respectively disposed near the first and second windows of the atomizer chamber to respectively have opaque plates that each have at least three apertures positioned relative to one another to define optical paths of the probe beam, first and second pump beams, and signal beam. The probe beam may form an acute angle of less than about 20 degrees with the first pump beam and be outside a plane defined by the first and second pump beams. The apertures in each alignment template are sized to make a volume formed by the overlapped probe beam, first and second pump beams less than a volume occupied by the sample vapor within the atomizer chamber. The first and second alignment templates may be symmetrically located with respect to a location where the probe beam, the first and second pump beams overlap and a pattern of the apertures in the first alignment template is a center reverse image of a pattern of the apertures in the second alignment template.

This disclosure also discloses a method for performing spectroscopic measurements by using optical nonlinear four-wave mixing. First, a sample solution is prepared to include particles of a sample to be measured. Second, the sample solution is injected into an atomizer chamber of a gas-phase atomizer to vaporize the sample solution and to produce a sample vapor. Next, a probe beam, a first pump beam, and a second pump beam are produced to be coherent with one another and are in resonance with an absorption transition in

the sample. Two alignment templates are placed on both sides of the atomizer chamber to align the input beams and to block background light in the path of the signal beam. Each template has at least three apertures to align the probe beam, the first pump beam, and the second pump beam to cross and overlap with one another in the sample vapor within the atomizer chamber to produce a signal beam by optical nonlinear four-wave mixing. The probe beam forms an acute angle of less than about 20 degrees with respect to the first pump beam and may be outside a plane defined by the first and second pump beams and the pump beam. Finally, the signal beam is detected to extract spectroscopic and chemical information of the sample.

The details of one or more embodiments of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 shows one embodiment of an optical spectrometer based on four-wave mixing.

FIG. 2 illustrates one configuration of the alignment templates that align the input beams to an atomizer chamber.

FIGS. 3, 4, and 5 show measured laser wave-mixing signals for Rb solutions by using the spectrometer shown in FIG. 1.

Like reference numbers and designations in the various drawings indicate like elements.

DETAILED DESCRIPTION

FIG. 1 schematically shows one embodiment 100 of a four-wave-mixing gas-phase spectroscopic analyzer. A laser 102, e.g., a diode laser, produces a tunable coherent beam 103. The laser 102 is tuned to a desired wavelength in resonance with a selected spectral line of a sample under measurement. A mirror 104 and a lens 106 guide the beam 103 to beam splitters 108 and 110. The beam splitter 108 reflects a portion of the beam 103 as a probe beam 111P and transmits the remaining part of the beam 103 to the beam splitter 110. The beam splitter 110 produces a forward pump beam 111F by reflection and a backward pump beam 111B by transmission. Mirrors 112, 114, and 116 are positioned to respectively guide the backward pump beam 111B, the probe beam 111P, and the forward pump beam 111F so that they cross and overlap with one another. The probe beam 111P and the forward pump beam 111F cross each other to form an acute angle. A gas-phase atomizer 130, e.g., a graphite furnace electrothermal atomizer, is used to produce a vapor sample in a location where the beams overlap. The atomizer 130 has a chamber with a top wall 132 and a bottom wall 134, and two side windows 136 and 138 for receiving optical signals. A sample is resolved in a solvent which is injected from an injection port 131 on the top wall 132 into the atomizer 130. The injected liquid drops to the bottom wall 134 and becomes vaporized. A computer 160 is included in the analyzer 100 to process and store signals from the photodetector 150. The computer 160 may also function as a system controller to control operations of the atomizer 130 or the laser 102.

The probe beam 111P, the forward pump beam 111F, and the backward pump beam 111B as shown form a three-dimensional, non-planar, four-wave-mixing configuration. The probe beam 111P is in a different plane than the plane defined by the pump beams 111F and 111B. When two input beams intersect at a small angle in an optical medium, constructive and destructive interferences of these beams form periodic modulation in the medium. Two of the three input beams write a grating inside the graphite furnace, and the third input beam scatters off the grating to produce the fourth coherent signal beam 111S. The difference between ground- and excited-state populations across the modulation or gratings depends on the excitation wavelength and the angle between the grating-writing beams. The signal beam 111S is generated in an optical path different from the probe beam 111P and can be directly collected by a photodetector 150 (e.g., a photodiode). A detection module 142 is also positioned relative to the furnace chamber 130 to receive the transmitted probe beam 132 and measures certain properties of the transmitted probe beam 132. For example, the detection module 142 may include a wavemeter to measure the wavelength of the probe beam for spectroscopic analysis.

Two alignment templates 120 and 122 are respectively placed on two sides of the furnace chamber 130 for pre-alignment of the three input beams 111P, 111F, and 111B. FIG. 2 shows one embodiment of the alignment templates 120 and 122. Each alignment template may be an opaque mask having at least three specially designed holes for transmitting beams. Thus, templates 120 and 122 also serve as spatial filters to prevent the scattered background light from reaching the detector 150. The templates 120 and 122 may be made by simply forming four small holes, one for each beam involved in a four-wave mixing process, in two thin aluminum plates. The pattern formed by the holes in one template is inversely symmetric with respect to the pattern formed by the holes in another template. In FIG. 2, the templates 122 and 120 have holes 210A, 220A, 230A, 240A and 210B, 220B, 230B, 240B, respectively. The templates 122 and 120 are positioned relative to the furnace chamber 130 so that holes 210A and 210B define the path of the forward pump 111F, holes 220A and 220B define the path of the probe 111P, holes 230A and 230B define the path of the signal beam 111S, and holes 240A and 240B define the path of the backward pump 111B. The positions of holes 230A and 230B can be determined based on given directions of beams 111F, 111B, and 111P according to the vector conservation requirement of the four-wave mixing process. The pump beams 111F and 111B may be opposing each other, where the beam 111F goes through the holes 210A and 210B and the beam 110B goes through the holes 240B and 240A. When the beams 111F and 111B are counter propagating, they go through the same pair of holes.

The positions of the templates 120 and 122 are fixed relative to each other for a desired four-wave mixing configuration. The chamber of the gas-phase atomizer 130 may be adjusted to change its position relative to the templates 120 and 122 so the laser beams can cross and overlap in a desired position within the chamber. The relative position of a hole relative to other holes in each template is selected so that the four beams 111F, 111B, 111P, and 111S overlap in a common volume with desired crossing angles with respect to one another at a desired spacing between two templates 120 and 122. The common volume is positioned within the furnace chamber 130 at where the sample vapor is located. Preferably, the forward pump beam 111F and the probe beam 111P form an acute crossing angle of less than about 20°, more preferably less than 10°, and most preferably, less than 5°. This crossing angle is important since it affects the sharpness of gratings formed in the sample, the grating periods, and the grating washout due to thermal motion. The dimension of the holes is selected to control the beam size and also determines the volume of the overlap region at a

given crossing angle between the beams 111F and 111P. The volume of the beam overlap region is adjustable and is usually smaller than the volume of the sample vapor produced in the furnace 130 so that the entire beam overlap volume is within the vapor volume. The dimension of the holes may be further adjusted to filter out the background noise in the signal beam 111S to achieve a desired signal-to-noise ratio.

Once the templates 120 and 122 are placed at their desired positions at both ends of the furnace 130, the optical components then can be pre-aligned. For example, the prealignment can be accomplished by using a visible alignment laser beam to produce four alignment beams to respectively trace the paths of beams 111F, 111B, 111P, and 111S. Thus, the first and second alignment templates 120 and 122 can be aligned in their desired positions relative to the chamber 130. Other beam guiding elements and the elements in the path of the signal beam 111S (e.g., detector 150) can also be aligned. Upon completion of the prealignment, the actual pump beams 111F and 111B and the probe beam 111P can be generated to trace the alignment beams. Therefore, implementation of templates 120 and 122 significantly simplifies the otherwise time-consuming optical alignment and optical maintenance of the system. This is especially important when using atomizers that do not fire continuously, and hence, the signal 111S is not continuously present. Thus, the optical alignment in general cannot be performed conveniently by using the generated four-wave mixing signal 111S during the transient period when the sample vapor exists.

Referring back to FIG. 1, the spectrometer 100 may also include a polarizer 145, an optical bandpass filter 146, and a lens 148 in the optical path of the signal beam 111S. These components and the photodetector 150 can be pre-aligned by using an alignment beam, preferably visible, to trace the path of the signal beam 111S. The optical filter 146 is configured to transmit light at the signal wavelength and block light at other wavelengths such as the white light emitted from the graphite furnace 130. The polarizer 145 is aligned to have its polarization substantially parallel to the signal polarization to provide polarization discrimination. The polarizer 145 may also be used to implement polarization modulated detection techniques to increase the signal-to-noise ratio in the signal detection. When all three input beams are vertically polarized, for example, the signal beam 111S is vertically polarized; when one of the three input beams 111F, 111B, and 111P is orthogonally polarized relative to the other two input beams, the signal beam 111S is orthogonally polarized. Polarization properties of laser wave mixing can be advantageously used for many important applications including sensitive circular dichroism spectroscopy. In addition, the polarizer 145 blocks randomly-polarized background light from reaching the detector 150.

The spectrometer 100 can be operated in either a continuous sampling mode or a pulsed sampling mode. In the continuous sampling mode, the laser 102 produces a continuous wave beam 103. Hence, the signal 111S is also continuous. A lock-in amplifier 152 and a chopper 154 can be used to modulate one of the input beams (e.g., the probe beam 111P) in order to increase the signal-to-noise ratio. When the laser 102 is a pulsed laser, analyzer 100 operates in the pulsed sampling mode and a "boxcar" averager may be used to replace the lock-in amplifier. In either mode of operation, the total intensity of the beams 111F, 111B, and 111P is preferably at or close to the saturation intensity of the sample to achieve an efficient four-wave mixing. The saturation intensity generally varies from one sample to another depending on properties of the excited atomic or molecular transition in the sample. When the total intensity is above the saturation intensity of a sample, the efficiency of the wave mixing decreases and other adverse effects arise, including power broadening of the spectral line that reduces the spectral resolution. In addition, the laser 102 may be configured to produce a narrow linewidth to achieve a high spectral resolution in the sub-Doppler range since the counter-propagating configuration of the pump beams 111F and 111B essentially eliminates Doppler broadening. Further, the frequency of the laser 102 may be stabilized to avoid mode hopping and to further improve the spectral resolution and the signal-to-noise ratio. Preferably, the linewidth of the laser 102 is stabilized at about a few MHZ (e.g., 1 MHz).

One implementation of the atomizer 130 is a graphite furnace electrothermal atomizer 130 for gas-phase studies at trace concentration levels. Graphite furnace detection limits are generally better than those of flame- and ICP-based conventional spectrometric methods. Graphite furnace also offers many advantages including convenient, fast, automated introduction of different types of samples, small sample size requirements, clean atomization environment, and effective use of matrices and modifiers to minimize interferences and optimize detection sensitivity. The graphite furnace electrothermal atomizer provides high sensitivity, small injection volumes, convenient sample introduction, automated programmable experimental controls, clean reducing atomization environment, and minimum background noise. The laser wave-mixing optical setup can be easily interfaced to many commercial graphite furnace systems for conventional atomic absorption spectrometers. The graphite furnace atomizer may be configured to operate based on drying, ashing and atomization stages. The drying stage evaporates the solvent, and the ashing stage further removes other remaining residues. Chemical matrix modification drastically helps remove unwanted residues and preserves the analytes for atomization in the final atomization stage. One chemical matrix modification uses a properly selected solvent or other chemical substance as an ionization suppressor to prevent atoms of molecules of a sample from ionizing during the drying stage. The ionization suppressor can ionize more readily than the analyte of interest, and hence, can flood the furnace-chamber 130 with electrons so that ionization of the analyte is reduced. The wave-mixing signal is generated during the atomization stage which may last a few seconds. An inert gas (e.g., the argon gas at 20 psi) may be used to flush out unwanted residues during these stages and thus prevent oxidation.

The properties of the laser 102 in the spectrometer 100 of FIG. 1, such as the type of the laser, the laser wavelength and the laser power, may vary depending on the type of sample solutions under measurement. For example, when a Rb vapor is studied, the laser 102 may be a laser diode emitting at 780 nm with a low output power (e.g., about 22 mW to produce 14 mW at the atomizer 130). The furnace chamber 130 may be a graphite furnace from a commercial graphite furnace system, e.g., Model GTA96 from Varian Australia Pty. Ltd. The laser diode may be cooled at 4.3° C. inside a temperature-controlled laser diode mount (e.g., Model LDM-4407 ILX Lightwave Corp.) and flushed with nitrogen gas in order to prevent condensation at low temperatures. The laser diode can be driven by a temperature controller (e.g., Model LDT-5910B from ILX Lightwave Corp.) and a low-noise current controller (e.g., Model LDX-3620 from ILX Lightwave Corp.). The laser wavelength is tunable around 780 nm by controlling the temperature and/or the

driving electrical current of the laser diode. To reduce the background noise and to prevent the graphite furnace power supply from affecting the laser stability, the current controller is operated by using a built-in low-noise battery instead of AC power. The laser diode wavelength is measured by using a high-resolution wavemeter (e.g., Model WA-20 from Burleigh Instruments). Laser power measurements are made using a low power laser detector (e.g., PM3 from Molectron).

The lens **106** is so positioned that its translational movement can change the beam waist of all three input beams **111F**, **111B**, and **111P** inside the chamber **130**, and thus, change the illuminated volume within the chamber **130**. The forward, backward, and probe beam intensities may be about 5.5 mW, 2.7 mW, and 6.0 mW, respectively. The laser power is distributed among the three input beams so that the intensity levels of the forward pump beam **111F** and the probe beam **111P** are stronger than the backward pump beam **111B**. The intensity of the probe beam **111P** may be modulated by using an optical chopper (e.g., SR540 from Stanford Research Systems). The photodetector **150** may be an amplified photodiode (e.g., PDA50 by Thorlabs) that is placed inside a Faraday cage to reduce electrical noise. The lock-in amplifier **152** may be a DSP lock-in amplifier SR810 by Stanford Research Systems and set at a time constant of 100 ms.

Rubidium stock solutions may be prepared to evaluate the performance of the above system by dissolving RbCl in 0.1% HNO_3 solutions (doubly deionized water), and then diluted to prepare ppt- and ppq-level analyte solutions. It is determined that 0.1% HNO_3 solvent may be used to achieve a high detection sensitivity for rubidium. The total injection volume within the atomizer may be about 20 μL (i.e., 10 μL analyte and 10 μL HNO_3 blank).

The rubidium D_2 resonance line $5s\ ^2S_{1/2}-5p\ ^2P_{3/2}$ at 780 nm has a relatively strong absorption oscillator strength at a value of 0.67. For the particular laser diode used, a current-temperature combination at about 4.2° C. and 63.5 mA is found to provide a tunable laser output across the Rb line with good stability and minimal mode hopping. The wavelength is tuned continuously by varying the driving current of the laser diode at a constant temperature of about 4.2° C. Using graphite furnace atomization the maximum absorption wavelength for Rb is monitored on a wavemeter at 12816.4 cm^{-1} (780.249 nm).

The wave-mixing signal peak generated by the graphite furnace is verified by injecting a few blanks and monitoring the blank peaks after each analyte injection. The blank is the same blank solvent matrix used to prepare the analyte solution so that the signal detected from a blank injection represents the background level. It is important to make certain that all memory effects are eliminated by firing a few blanks and monitoring the background level. If the blank peak is unusually high, a memory effect may be present due to the accumulation of analyte residues inside the graphite tube. Some memory effect can be observed when analyte concentration is high (e.g., greater than 10 parts-per-trillion) or when analyzing some elements of low volatility, especially if the atomization temperature and timing are not optimized to ensure complete elimination of analyte and matrix residues after each injection. At the very high analyte concentration range (i.e., >1 parts-per-million), it is especially important to use a high-temperature tube cleaning procedure between analyte runs to ensure complete elimination of analyte residues inside the graphite tube.

FIG. 3 shows measured laser wave-mixing peaks for a high-concentration 100 ppb Rb injection and the resulting memory effect. At this relatively high concentration level, at least five blank injections may be performed before the next analyte injection in order to eliminate all the residues. A tube flushing procedure ensures elimination of the memory effect as much as possible. The tall injection peaks are recorded when the input laser beams are reflected by the injector arm entering the graphite tube through the injector port.

FIG. 4 shows three peaks observed in a 10-parts-per-quadrillion Rb analyte with three blank peaks following each analyte injection. At this lower concentration level, the memory effect is relatively insignificant, and the analyte peaks are still consistently well above the blank peaks. Even at this low concentration range, three blank injections are performed after each analyte injection to ensure that no residues remain from the previous analyte injection. A new graphite tube is used when changing to a new concentration range. For 1 ppq Rb injections, memory effects no longer exist and blank peaks are so small that they are buried under the baseline noise level. At the 1 ppq concentration level, the analyte amount injected is so minute that the normal furnace firing program and the argon flush manage to eliminate any minute analyte residue, and hence, subsequent blank firings show negligible blank peaks.

FIG. 5 shows the four-wave-mixing signal peak at 0.1 ppq concentration. This corresponds to a preliminary "injected" mass detection limit of 0.07 ppq, 0.7 attogram or 8 zeptomole (at S/N 2) based on 20 μL injection (i.e., 10 μL analyte, 10 μL blank). Assuming that approximately 15% is lost during the drying, ashing, and atomization stages, and that the laser probe volume is 1% of that of the total analyte population volume produced, one can estimate that only about 60 Rb atoms are present inside the laser cross overlap volume between the two beams that write the wave-mixing gratings. The number of Rb atoms present inside the laser probe volume is even smaller when the short residence time during the atomization stage is considered.

The spectrum in FIG. 5 is recorded by the lock-in amplifier using a 2.5 kHz amplitude modulation frequency with a photodiode and an optical chopper. The preliminary detection limits of 0.07 ppq, 0.7 attogram and 8 zeptomole are encouraging especially since the graphite furnace is not a continuously firing atomizer with a stable atom population, and it has only an atomization time window of about 2 seconds. The detection sensitivity may be improved by other detection techniques such as modulating the wavelength of the laser **102** to turn the wavelength of the beams on and off the resonance wavelength to modulate the intensity of the signal beam or by modulating the polarization of one input beam to modulate the polarization of the signal beam.

Some procedures are found to be effective in enhancing the signal-to-noise ratio in the system. For example, a grounded Faraday cage around the photodetector may be used to substantially reduce the baseline noise level. The use of Teflon wedge O-rings to seal the graphite furnace windows can also significantly reduce the optical background noise. The windows are tilted at an angle to prevent the reflected input beams from retracing back. Micro spatial filters can be used in addition to the alignment templates **120** and **122** to significantly enhance the signal-to-noise ratio since the signal beam (i.e., a laser beam) can be focused very tightly to go through the micro apertures while the background noise is blocked. A wavelength filter may also be used to improve the noise filtering in detecting the signal.

The signal-to-noise ratio can be also improved by positioning the laser overlap probe volume in the top third of the graphite tube (i.e., closer to the top wall of the tube).

Although the wave-mixing signal strength is the same when probing the lower section or the upper section of the graphite tube, the noise levels during the drying and ashing stages are lower when the laser probe volume is positioned in the upper third section of the graphite tube. Since the vaporization of the analyte liquid occurs at the bottom wall of the graphite tube, clusters of small liquid drops produced from the vaporization of the solvent are concentrated near the bottom wall and can scatter the laser beams. Hence, when the laser probe volume is placed near the tube bottom, the scattered background light level is higher than the background light when the laser probe volume is placed near the top of the tube. The position of the overlap probe volume of all the input beams can be adjusted within the tube to improve the signal-to-noise ratio by lowering or raising the graphite tube without disturbing the rest of the laser alignment.

Different types of graphite tubes available commercially have different advantages. Both partition-type and platform-type tubes are found to be effective in the analyzer **100** shown in FIG. **1**. The design of the platform-type graphite tube slightly restricts the alignment of the input beams in our non-planar three-dimensional wave-mixing setup. Hence, partition-type graphite tubes with their more open design are used throughout this work for Rb measurements. Proper uses of the graphite tube gas flow and the matrix composition are important since they ensure optimal graphite furnace atomization conditions, removal of analyte and matrix residues, and prevention of rapid tube oxidation.

The four-wave-mixing signal in general has a cubic dependence on the input laser power. The signal strength, and hence, the detection sensitivity can be further enhanced by using higher input beam intensities up to about the saturation intensity. The four-wave-mixing signal also has a quadratic dependence on absorption coefficient. For analytes with low absorption coefficients, the wave-mixing detection sensitivity is still comparable to or better than those of conventional laser methods because of the nonlinear signal properties such as cubic power dependence, virtually 100% optical collection efficiency, and the laser-like coherence properties of the signal beam. For example, unlike laser-induced fluorescence methods where the signal is a small fraction of a widely diffused fluorescence signal laser, the wave-mixing signal is a collimated coherent laser-like beam and hence nearly the entire signal beam can be directed into a photodetector. Furthermore, since wave mixing is an absorption method, both fluorescing and non fluorescing analytes can be measured.

Although only a preferred embodiment has been described, various modifications may be made. For example, different types of diode lasers or other types of lasers (e.g., fiber lasers) may be used. Different types of graphite furnace, various gas-phase, liquid-phase and solid-phase sample cells may also be used. The graphite tube may not be a closed cell but has an opening. An inductively-coupled-plasma (ICP) chamber may be used. Further, optical fibers may be used to transmit the pump and probe beams from the laser to the graphite furnace. These and others are intended to be within the scope of the following claims.

What is claimed is:

1. A device for optical nonlinear four-wave mixing, comprising:

(1) a gas-phase atomizer having an atomizer chamber with a bottom wall and a top wall and operable to vaporize a sample solution received from the bottom wall to produce a sample vapor, the atomizer chamber having a first optical window to receive a probe beam and a first pump beam, and a second optical window opposing the first optical window to receive a second pump beam, wherein the probe beam, the first and second pump beams overlap in the sample vapor to produce a signal beam through a four-wave mixing process in the sample vapor;

(2) first and second alignment templates each having at least three apertures formed on an opaque plate and respectively disposed near the first and second windows of the atomizer chamber to define optical paths for the probe beam, first and second pump beams, and signal beam so that the probe beam forms an acute angle of less than about 20 degrees with the first pump beam and is outside a plane defined by the first and second pump beams, and the probe beam, the first and second pump beams overlap in a location within the atomizer chamber closer to the top wall than the bottom wall, wherein each optical path is determined by one aperture in the first alignment template and another aperture in the second template and the apertures in each alignment template are sized to make a volume formed by the overlapped probe beam, first and second pump beams less than a volume occupied by the sample vapor; and

(3) a laser tunable to generate a laser beam at a desired wavelength corresponding to an absorption line in the sample vapor and to produce a total laser intensity approximately equal to a saturation intensity of the absorption line.

2. The device as in claim **1**, wherein the first and second alignment templates are symmetrically located with respect to a location where the probe beam, the first and second pump beams overlap and a pattern of the apertures in the first alignment template is a center reverse image of a pattern of the apertures in the second alignment template.

3. The device as in claim **1**, further comprising a spatial filter in each optical path to reduce background noise in the signal beam.

4. The device as in claim **1**, wherein the laser comprises a diode laser.

5. The device as in claim **4**, wherein the diode laser is tunable by adjusting at least one of a temperature and a driving electrical current of the diode laser.

6. The device as in claim **1**, wherein the laser comprises a fiber laser.

7. The device as in claim **1**, further comprising a photo detector disposed in the optical path of the signal beam to convert the signal beam into an electrical signal and a polarizer having a polarization direction substantially parallel to a polarization of the signal beam to filter light received by the photo detector.

8. The device as in claim **1**, wherein the atomizer chamber includes a graphite furnace.

9. The device as in claim **1**, wherein the atomizer chamber is configured to vaporize the sample solution by an inductively coupled plasma process.

10. A method for performing spectroscopic measurements by using optical nonlinear four-wave mixing, comprising:

(1) preparing a sample solution that includes particles of a sample to be measured;

(2) injecting the sample solution into an atomizer chamber of a gas-phase atomizer to vaporize the sample solution and to produce a sample vapor;

(3) producing a probe beam, a first pump beam and a second pump beam that are coherent with one another and are in resonance with an absorption transition in the sample;

(4) overlapping the probe beam, the first pump beam, and the second pump beam in the sample vapor within the atomizer chamber to produce a signal beam by optical nonlinear four-wave mixing;

(5) respectively placing first and second alignment templates each having at least three apertures formed on an opaque plate on both sides of the atomizer chamber to define optical paths for the probe beam, first and second pump beams, and signal beam so that the probe beam forms an acute angle of less than about 20 degrees with the first pump beam and is outside a plane defined by the first and second pump beams, and the probe beam, the first and second pump beams overlap in a location within the atomizer chamber closer to the top wall than the bottom wall; and

(6) detecting the signal beam to extract spectroscopic information of the sample.

11. The method as in claim 10, further comprising configuring the first and second alignment templates to make a volume formed by the overlapped probe beam, first and second pump beams less than a volume occupied by the sample vapor.

12. The method as in claim 10, further comprising using a laser to produce the probe beam, first and second pump beams.

13. The method as in claim 12, wherein the laser includes a diode laser and is tunable by adjusting at least one of a temperature and a driving electrical current of the diode laser.

14. The method as in claim 12, wherein the laser includes a fiber laser.

15. The method as in claim 10, further comprising using a polarizer that has a polarization direction substantially parallel to a polarization of the signal beam to reduce background noise in detection the signal beam.

16. The method as in claim 10, further comprising injecting a blank solution into the atomizer chamber that is used for the sample solution and does not include particles of the sample before injecting the sample solution, and detecting a signal beam associated with the blank solution as background noise in detecting the signal beam obtained from the sample solution.

17. The method as in claim 10, wherein the preparation of the sample solution includes adding an ionization suppressor in the sample solution to reduce ionization of particles of the sample during vaporization.

18. The method as in claim 10, further comprising keeping a total intensity of the probe beam, first and second pump beams approximately equal to or less than a saturation intensity of the sample.

19. The method as in claim 10, further comprising vaporizing the sample solution by a inductively coupled plasma process.

20. A method for performing spectroscopic measurements by using optical nonlinear four-wave mixing, comprising:

(1) preparing a sample solution that includes particles of a sample to be measured;

(2) injecting the sample solution into an atomizer chamber of a gas-phase atomizer to vaporize the sample solution and to produce a sample vapor;

(3) respectively placing first and second alignment templates each having at least three apertures formed on an opaque plate on both sides of the atomizer chamber to define optical paths for a probe beam, first and second pump beams, and a signal beam so that the probe beam forms an acute angle of less than about 20 degrees with the first pump beam and is outside a plane defined by the first and second pump beams, and the probe beam, the first and second pump beams overlap in a location within the atomizer chamber closer to the top wall than the bottom wall,

(4) using an alignment optical beam to produce a probe alignment beam which traces the probe beam, a first pump alignment beam which traces the first pump beam, a second pump alignment beam which traces the second pump beam, and a signal alignment beam which traces the signal beam, to align the first and second alignment templates and other beam guiding elements;

(5) using a laser to produce and direct the probe beam, first and second pump beams in their respective optical paths determined by the first and second alignment templates; and

(6) detecting the signal beam to extract spectroscopic information of the sample.

21. The method as in claim 20, further comprising configuring the first and second alignment templates to make a volume formed by the overlapped probe beam, first and second pump beams less than a volume occupied by the sample vapor.

22. The method as in claim 20, further comprising using a polarizer that has a polarization direction substantially parallel to a polarization of the signal beam to reduce background noise in detection the signal beam.

23. The method as in claim 20, further comprising injecting a blank solution into the atomizer chamber that is used for the sample solution and does not include particles of the sample before injecting the sample solution and detecting a signal beam associated with the blank solution as background noise in detecting the signal beam obtained from the sample solution.

24. The method as in claim 20, wherein the preparation of the sample solution includes adding an ionization suppressor in the sample solution to reduce ionization of particles of the sample during vaporization.

25. The method as in claim 20, further comprising keeping a total intensity of the probe beam, first and second pump beams approximately equal to or less than a saturation intensity of the sample.

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Tong

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[54] **DETECTING ANALYTE LIGHT ABSORPTION UTILIZING DEGENERATE FOUR WAVE MIXING**

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[58] Field of Search 356/432, 433, 356/434, 436, 437, 440, 491, 442, 335-342, 345, 346

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Primary Examiner—Frank Gonzalez
Assistant Examiner—Jason D. Eisenberg
Attorney, Agent, or Firm—Fish & Richardson P.C.

[57] **ABSTRACT**

A method and apparatus using either two or three input laser beams in a nonlinear degenerate four-wave mixing arrangement for ultrasensative analytical measurements of an analyte. In accordance with a first embodiment of the present invention, a two input beam F-D4WM arrangement is used to generate two phase conjugate signal beams. The input beams are narrowly focused to intersect within a very small volume of an analyte. The analyte may be in any physical state (e.g., liquid, solid, or gas). The intensity of the signal beam is used to detect trace concentrations of particular substances. The beam spot of each of the input beams can be focused to less than 34 μm, thus allowing the present invention to directly focus the input beams within a capillary tube of a HPCE or a column of a HPLC system. In accordance with the second embodiment of the present invention, the input laser beams of a F-D4WM method are directed to points on a lens by immobilized fiber optic cables. In accordance with a third embodiment of the present invention, a three input beam B-D4WM method is used in which the three input laser beams are directed by an immobilized fiber optic cable.

38 Claims, 11 Drawing Sheets

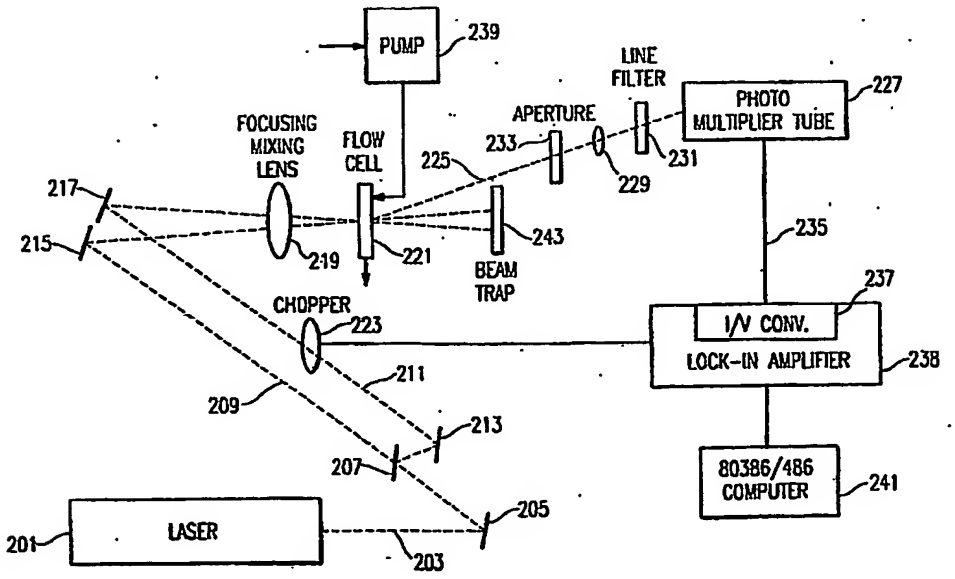
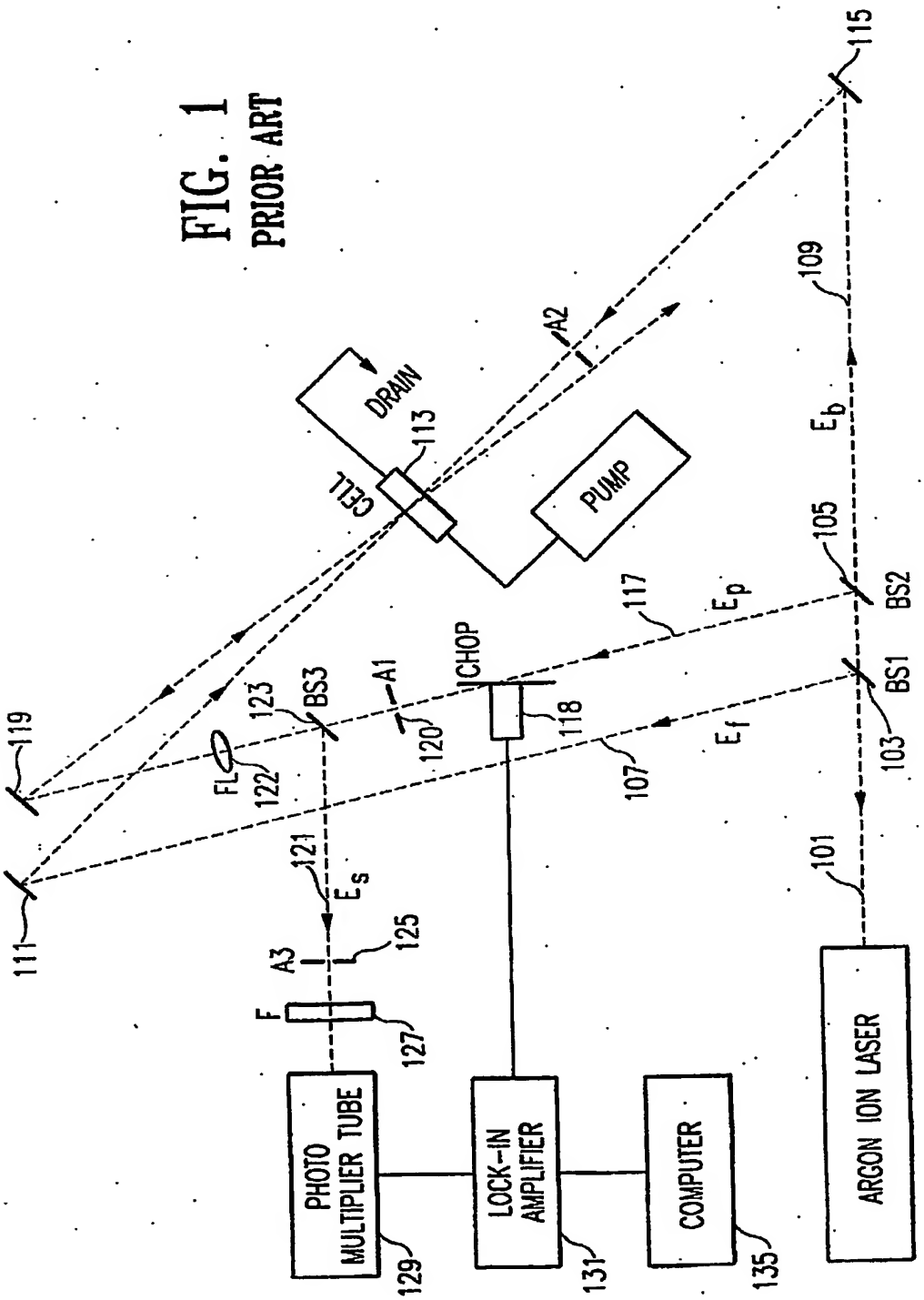


FIG. 1
PRIOR ART



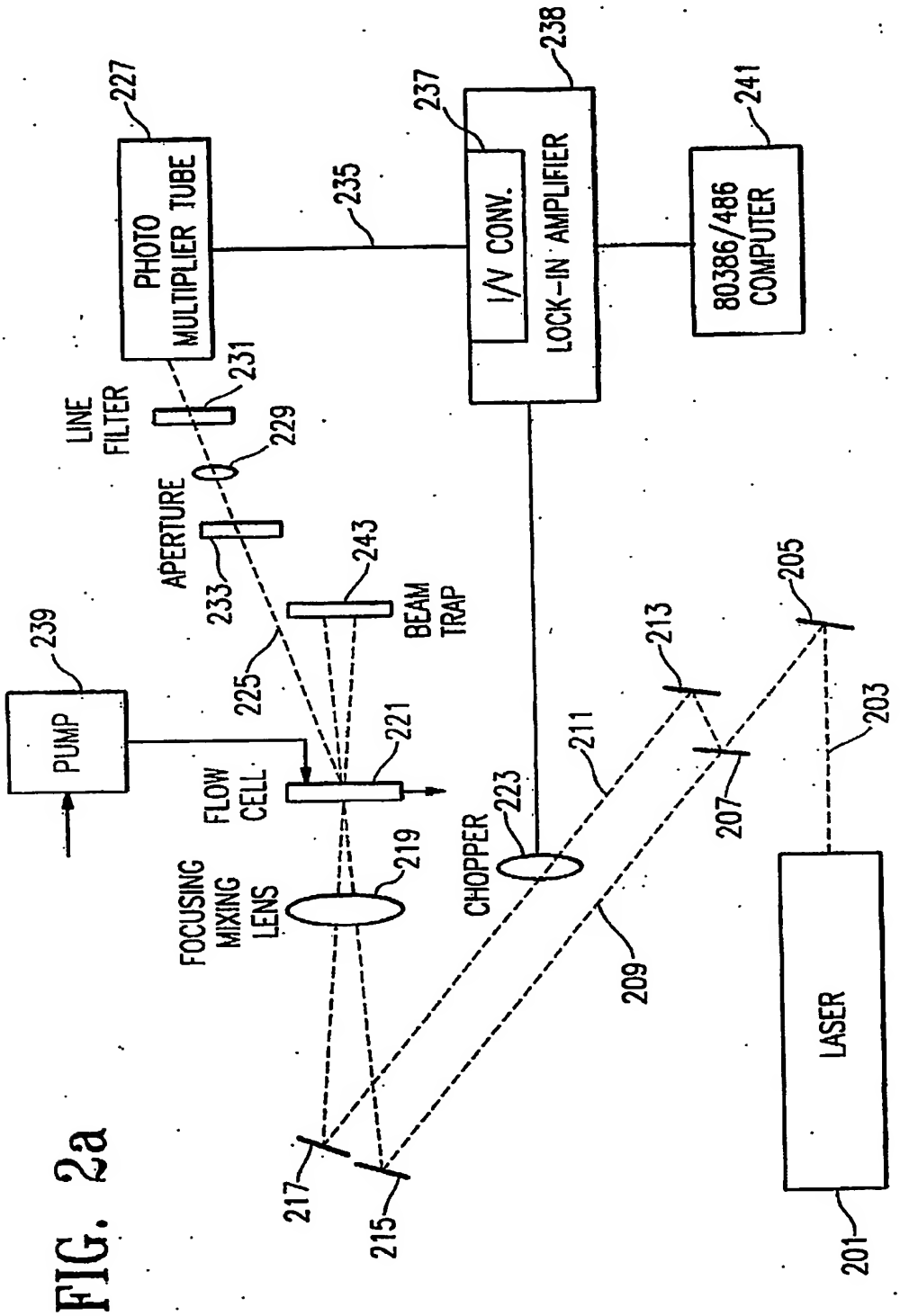


PHOTO MULTIPLIER TUBE
227
235
I/V CONV.
LOCK-IN AMPLIFIER
237
238
80386/486 COMPUTER
241
LINE FILTER
231
229
APERTURE
233
243
BEAM TRAP
239
PUMP
225
221
FLOW CELL
223
CHOPPER
211
213
205
203
207
219
FOCUSING MIXING LENS
209
LASER
217
215
201

FIG. 2a

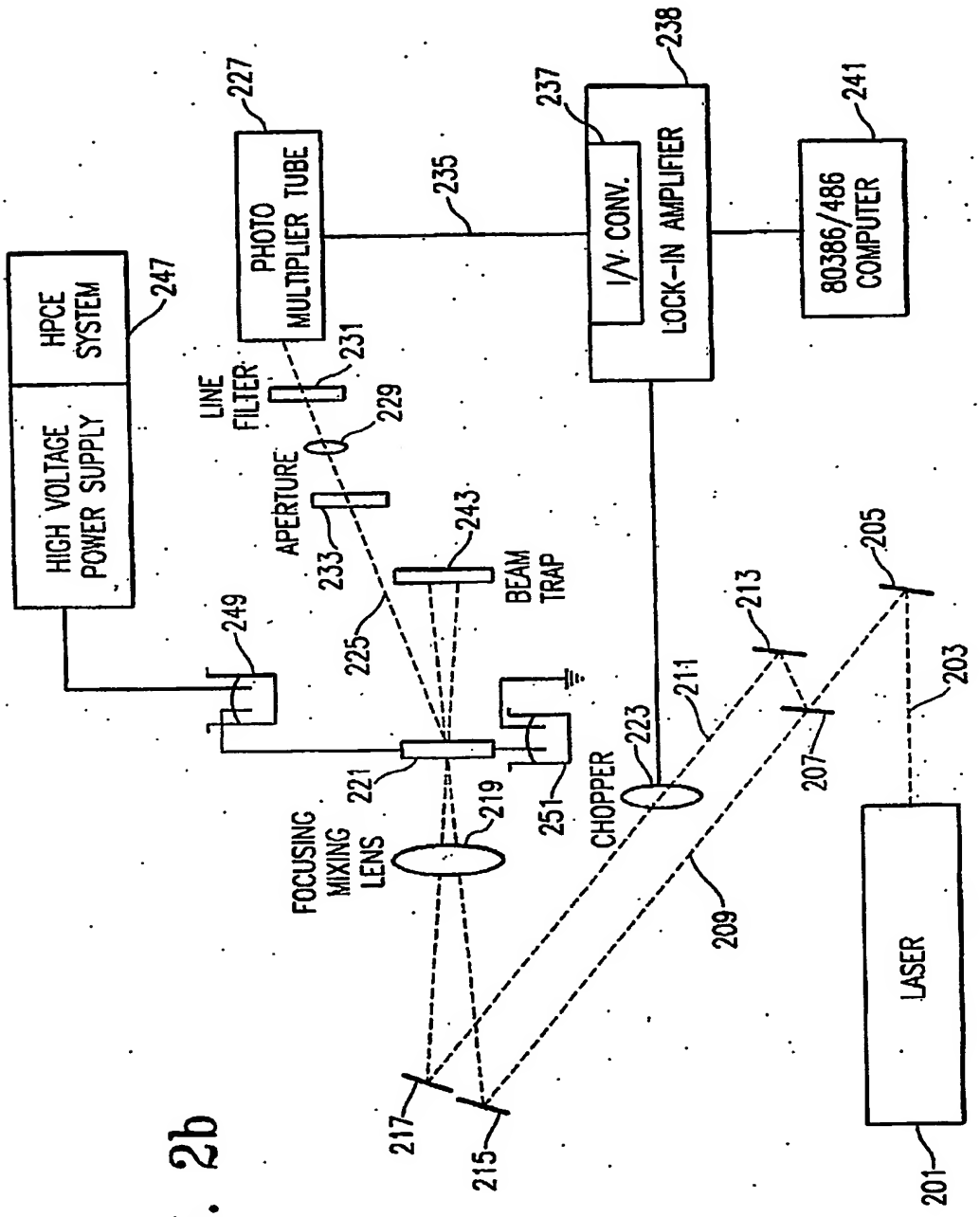

HPCE SYSTEM
HIGH VOLTAGE POWER SUPPLY
247
PHOTO MULTIPLIER TUBE
227
LINE FILTER
231
APERTURE
229
233
BEAM TRAP
243
225
249
221
219
FOCUSING MIXING LENS
251
CHOPPER
223
235
I/V CONV.
LOCK-IN AMPLIFIER
237
238
80386/486 COMPUTER
241
211
213
205
203
207
209
LASER
201
217
215

FIG. 2b

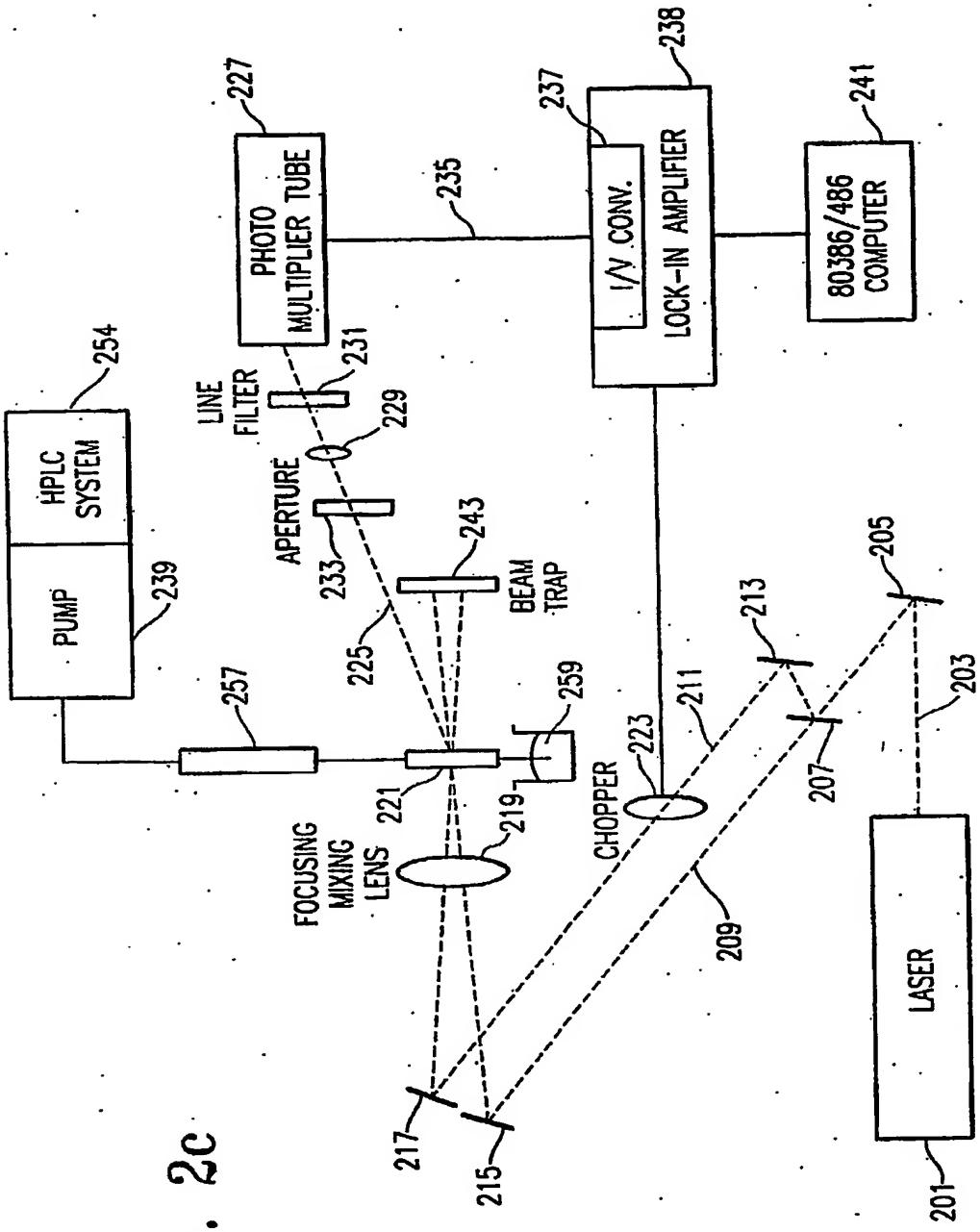

HPLC SYSTEM
PUMP
254
239
257
LINE FILTER
231
APERTURE
233
229
225
243
BEAM TRAP
259
221
219
223
CHOPPER
211
213
205
203
207
209
217
215
201
FOCUSING MIXING LENS
227
PHOTO MULTIPLIER TUBE
235
237
I/V CONV.
LOCK-IN AMPLIFIER
238
241
80386/486 COMPUTER
LASER

FIG. 2c

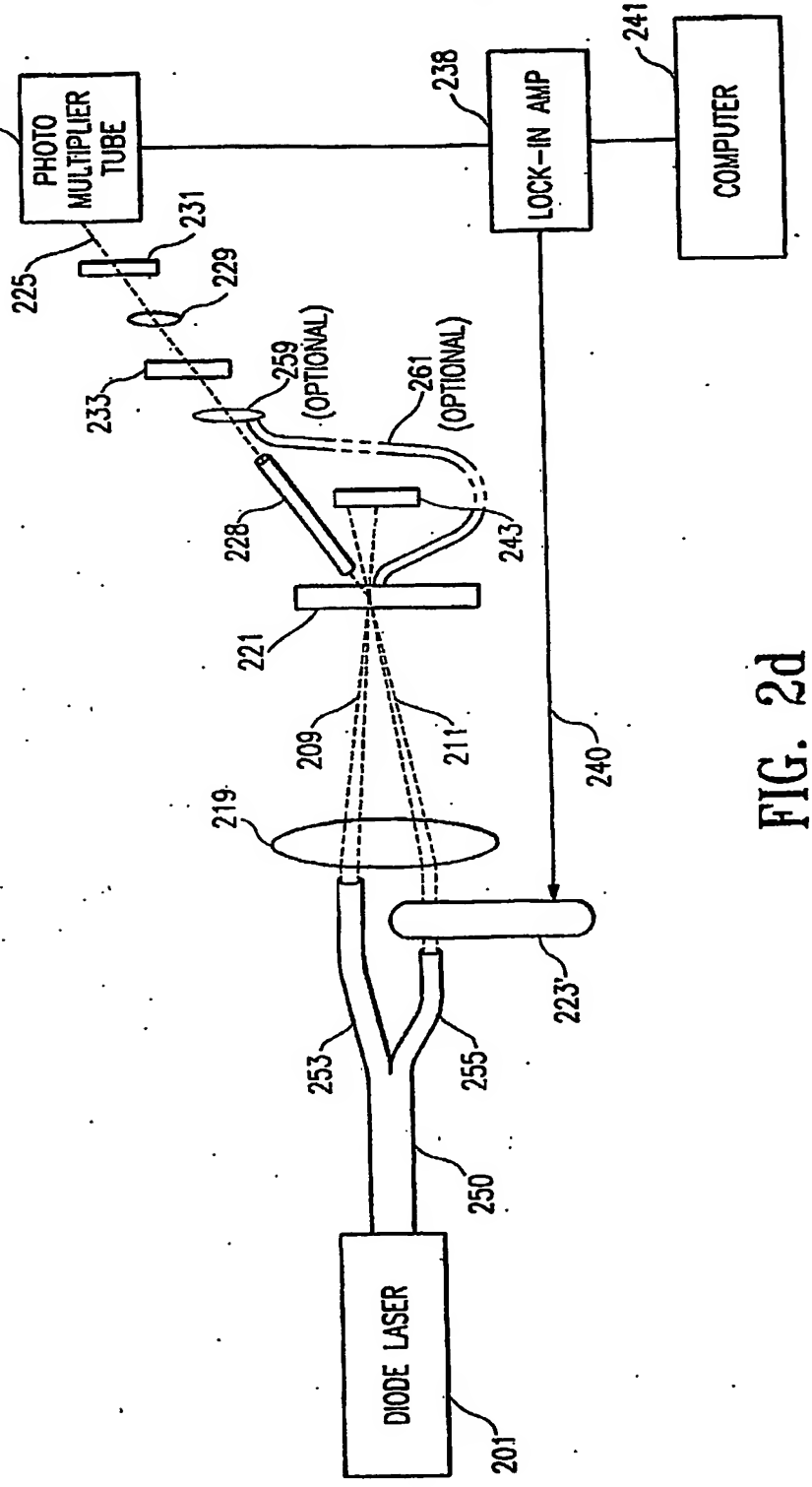

227
PHOTO MULTIPLIER TUBE
238
LOCK-IN AMP
241
COMPUTER
231
225
229
233
259 (OPTIONAL)
261 (OPTIONAL)
228
243
221
209
211
240
219
223'
253
255
250
DIODE LASER
201

FIG. 2d

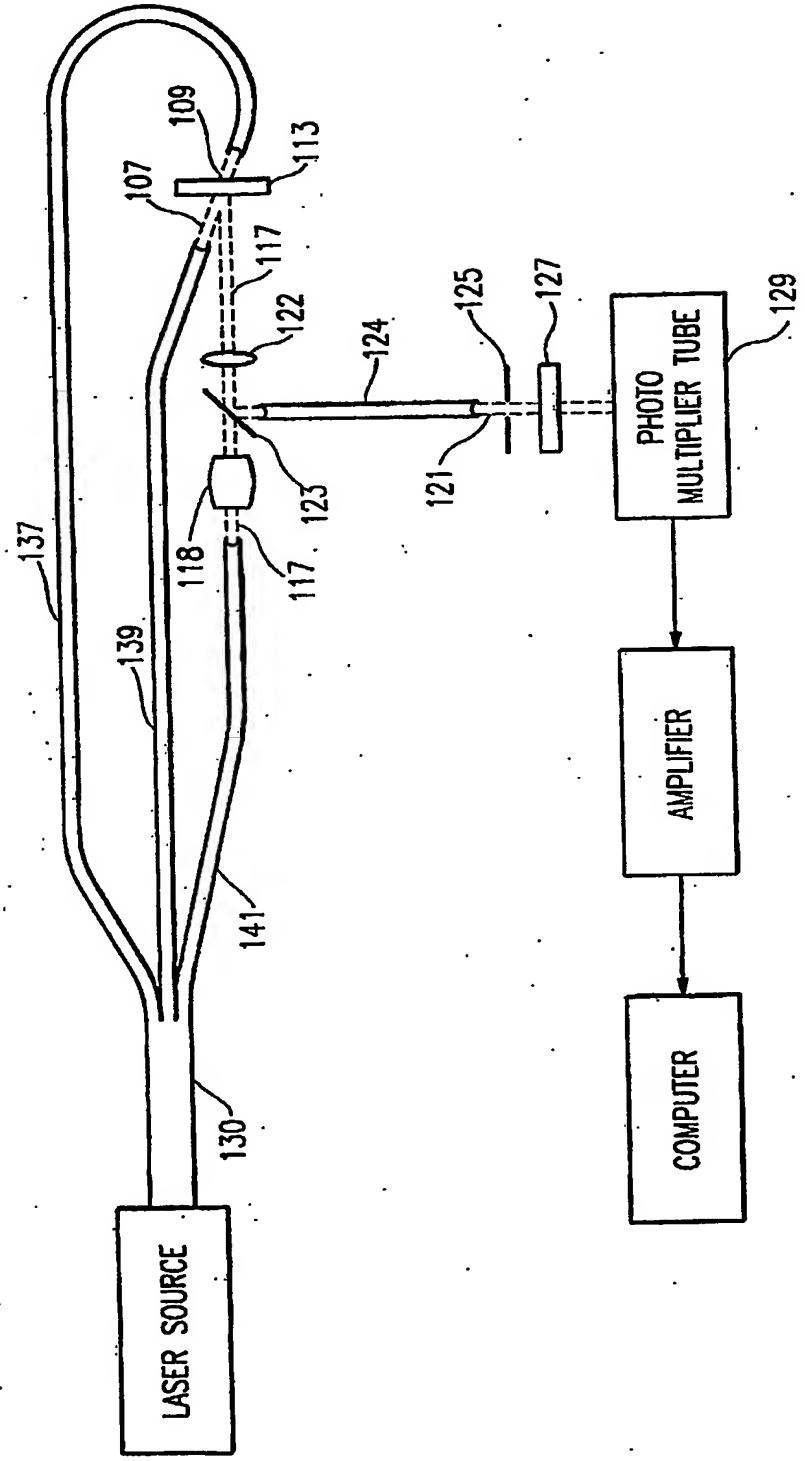

109
113
107
117
122
124
125
127
129
PHOTO MULTIPLIER TUBE
121
123
118
117
137
139
141
130
AMPLIFIER
COMPUTER
LASER SOURCE

FIG. 2e

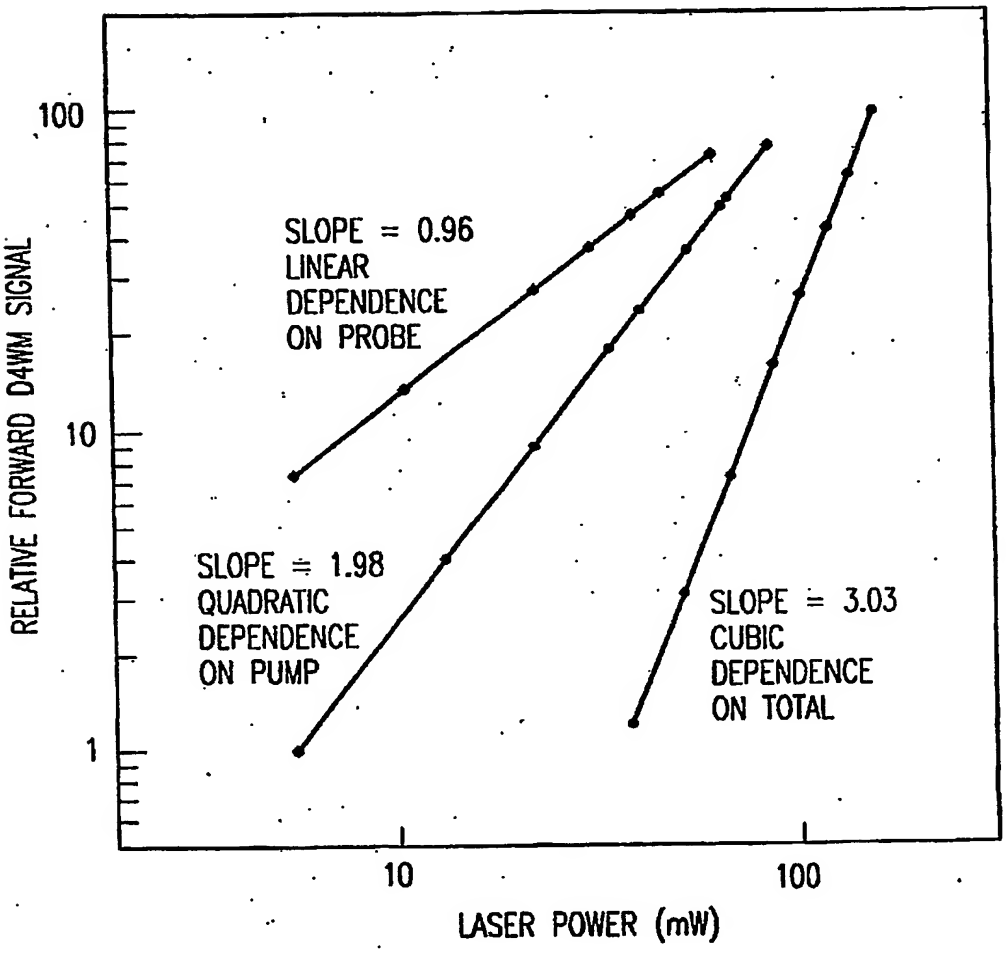


FIG. 3

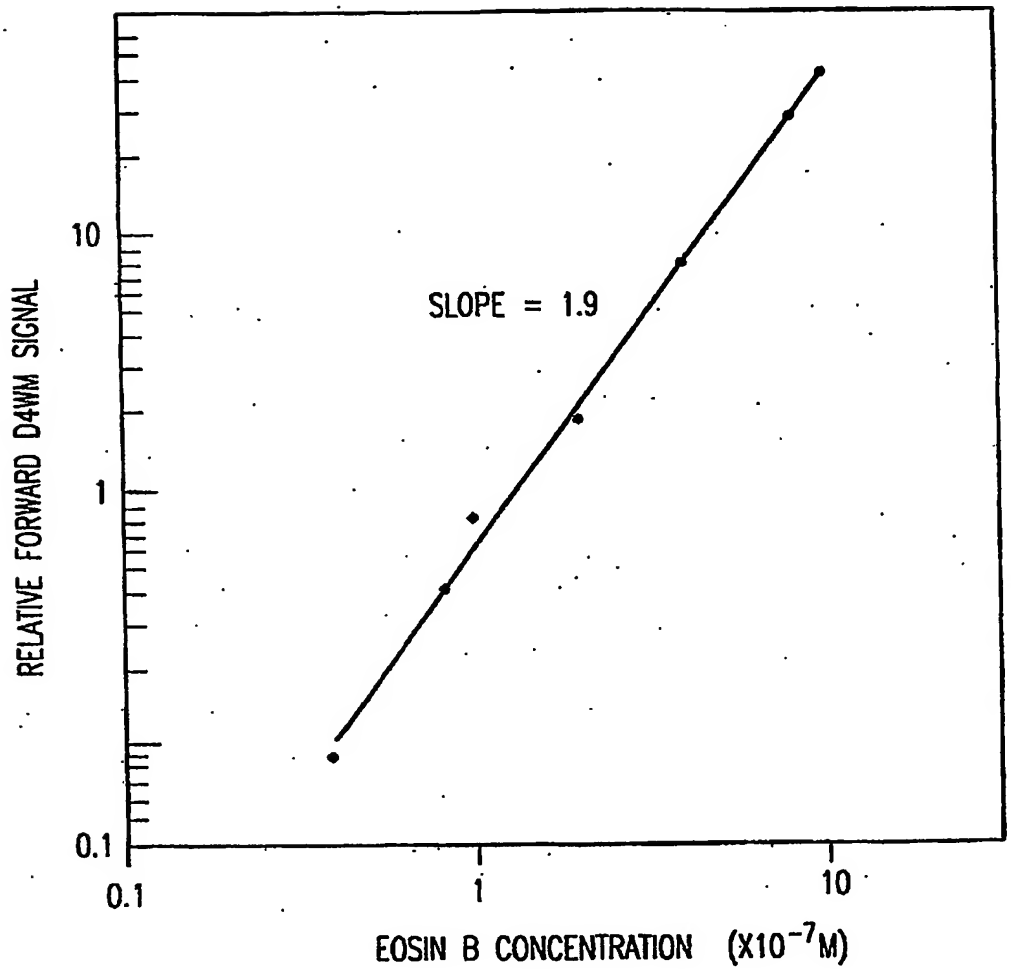


FIG. 4

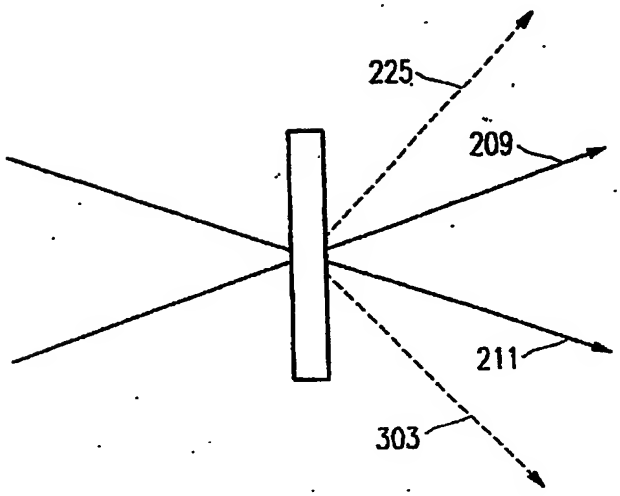


FIG. 5a

$$\Delta K = K_2 + K_3 - 2K_1$$

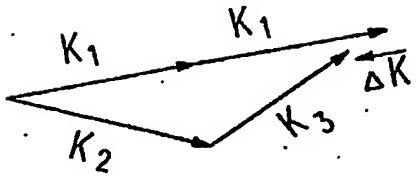


FIG. 5b

$$\Delta K = K_1 + K_4 - 2K_2$$

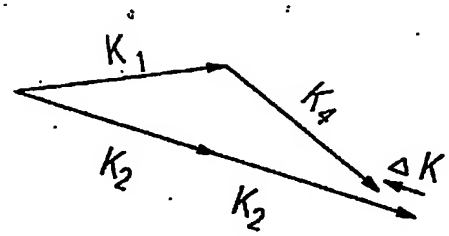


FIG. 5c

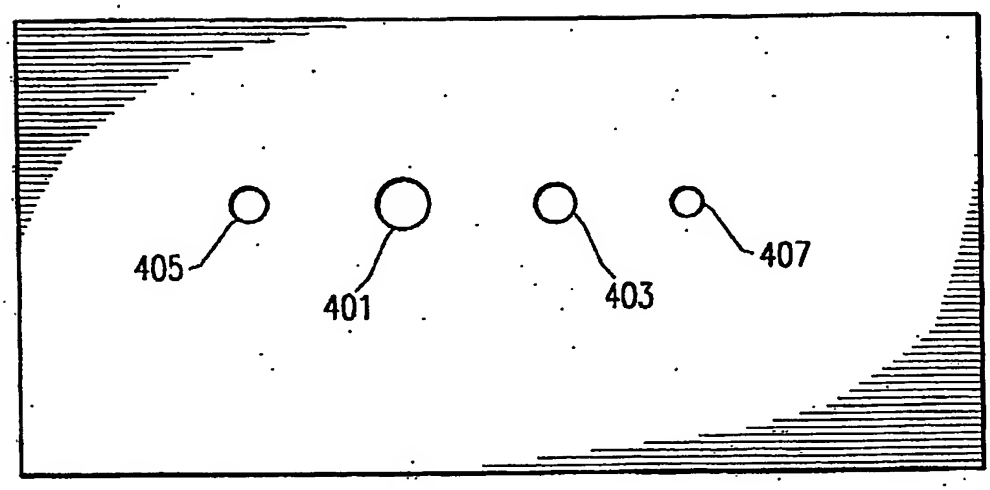


FIG. 6

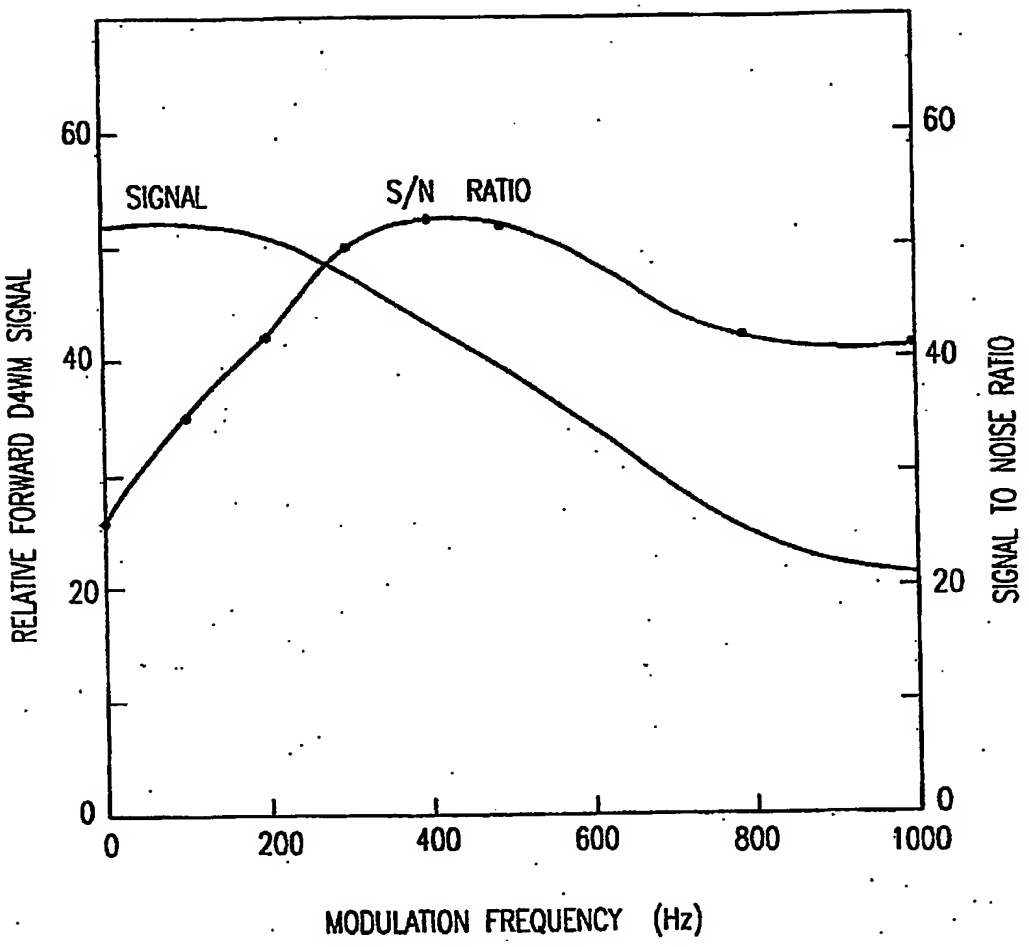

SIGNAL
S/N RATIO
RELATIVE FORWARD D4WM SIGNAL
SIGNAL TO NOISE RATIO
MODULATION FREQUENCY (Hz)
0
20
40
60
0
200
400
600
800
1000

FIG. 7

DETECTING ANALYTE LIGHT ABSORPTION UTILIZING DEGENERATE FOUR WAVE MIXING

This is a continuation of application Ser. No. 08/181,676, filed Jan. 13, 1994, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method and apparatus for nonlinear laser spectroscopy, and more particularly, to a method and apparatus using two or three input laser beams in a nonlinear four-wave mixing setup for ultrasensative analytical measurements of an analyte.

2. Description of Related Art

Research has taken place with regard to degenerate four-wave mixing (D4WM) techniques using a "backward-scattering" degenerate four-wave mixing (B-D4WM) optical setup for analytical measurements in discharge atomizers (see Tong, W. G., Chen, D. A., Appl. Spectrosc. 1987, 41, 586–590), flame atomizers (see Tong, W. G., Andrews, J. M., Wu, Z., Anal Chem. 1987, 59, 896–899), and room-temperature flow cells (see Wu, Z., Tong, W. G., Anal Chem. 1989, 61, 998–1001). In particular, the inventor of the present invention has described the use of B-D4WM to detect trace-concentrations of an analyte, such as eosin B dissolved in ethanol. In such an application of B-D4WM, two counter-propagating pump beams and a probe beam are mixed inside a nonlinear medium, and a phase-conjugate signal beam is generated via one of the four-wave mixing mechanisms. FIG. 1 illustrates the set-up used for detecting trace-concentrations in this manner. A laser beam **101** is generated by an argon ion laser. The laser beam is split by a first beam splitter **103**, such that a first portion of the laser beam **101** forms the forward pump beam **107**, and a second portion of the laser beam **101** forms the backward pump beam **109**. The forward pump beam **107** is reflected by a reflector **111** which directs the forward bump beam toward an analyte cell **113**. The analyte cell contains a volume of an analyte. The backward pump beam **109** is reflected by a second reflector **115** toward the analyte cell in the opposite direction from the forward pump beam **107**. A portion **117** of the laser beam that passes through the first beam splitter **103** is reflected by the second beam splitter **105**. The second portion of the laser beam is the probe beam **117**. The probe beam **117** is reflected by the second beam splitter **105** through an amplitude modulation device **118** (such as a mechanical chopper), an aperture **120**, a third beam splitter **123**, and a lens **122**, to a reflector **119**. The reflector directs the beam **117** to the point in the analyte cell **113** to which both the forward pump beam and the backward pump beam **109** are directed. Generation of an optical phase conjugate beam **121** by D4WM in an absorbing liquid sample of an analyte contained within the analyte cell **113** results from formation of spatial gratings due to a thermally induced refractive index change in the nonlinear medium of the sample. The phase conjugate reflectivity can be described as:

$$R=I_p/I_b=f^2Q^2I_pI_b\exp(-\alpha L)[1-\exp(-\alpha L/\cos\Theta)]^2G(t_D)$$

Where:

I_p, i_p, I_p, and I_b=the beam intensity of the conjugate signal beam **121**, probe beam **117**, forward pump beam **107**, and backward pump beam **109**, respectively;

f=the fraction of absorbed light energy converted into heat, and is inversely proportional to the quantum efficiency of fluorescence of the analyte;

α=the absorption coefficient;

L=the sample path length;

Θ=is the angle between the forward pump beam **107** and the probe beams **117**;

$Q=[2n\ (dn/dt)_p]/(\lambda\rho_oC_p)$;

$(dn/dt)_p$=the change of refractive index due to temperature change at constant pressure,

ρ_o=the equilibrium solvent density;

C_p=the specific heat at constant pressure,;

λ=the wavelength of the excitation laser source;

$G(t_D)$=thermal grating evolution and depends on the thermalization time and thermal diffusion time constant for the analyte molecules in the solution.

The above equation can be simplified to:

$$I_p=Q^2f^2(\alpha L/\cos\Theta)^2$$

by assuming that the absorption of the solution is small and the sample path length is short. Therefore, according to this formula, the intensity of the optical phase conjugate signal is proportional to the square of the absorption coefficient, and hence to the analyte concentration. Thus, by accurately measuring the intensity of the optical phase conjugate signal generated by incident light defracted off the spatial gratings generated in the sample in the analyte cell **113**, the concentration of the analyte can be determined.

The signal beam **121** is transmitted back along the path of the input beam **117**, and is reflected by the third beam splitter **123** toward an aperture **125**, a filter **127**, and a photomultiplier tube **129**. The output of the photomultiplier tube is coupled to a lock-in amplifier **131**, which filters out amplitude variations that occur at frequencies other than the frequency of the chopper **118** and out of phase with the chopper. The output of the lock-in amplifier **131** is then coupled to a computer **135** comprising an analog to digital converter. The computer **135** processes intensity information to determine the concentration of the analyte that was present in the sample cell **113**.

This method for measuring concentrations has a detection limitation that makes it useful for detecting trace amounts of a substance, such as eosin B which is used in many areas, including protein labeling, and artificial food coloring. However, the amount of analyte that must be provided within the sample cell of such detectors is greater than is desireable in many circumstances. For example, when used with a capillary electrophoresis system, the laser beams are very difficult to focus within the very small confines of capillary tubes typically used. Furthermore, the physical set-up is difficult to align due to the fact that three beams which reflect off of five surfaces must be aligned to cause a phase conjugate signal to be generated. Furthermore, it would be desireable to reduce the laser power requirements and even further decrease the mass detection limitations of the system. Still further, it would be desirable for the laser probe volume to be reduced with shorter analyte absorption path length.

The present invention provides a method and apparatus which has lower mass detection limitations than the prior art, requires less laser power, is much easier to align, provides a means by which the laser beams used can be focused and mixed with a single lens within a sufficiently small area to allow detection directly within the capillary tube of a capillary electrophoresis system, and allows virtually any substance to be analyzed.

SUMMARY OF THE INVENTION

The present invention is a method and apparatus using either two or three input laser beams in a nonlinear degen-

erate four-wave mixing arrangement for ultrasensitive analytical measurements of an analyte. In accordance with a first embodiment of the present invention, a two input beam forward-scattering degenerate four-wave mixing arrangement is used to generate two phase conjugate signal beams. Constructive interference between the two input beams creates a dynamic grating at a point at which the two input beams intersect. In accordance with the first embodiment of the present invention, the input beams are narrowly focused to intersect within a very small volume of an analyte. The analyte may be in any physical state (e.g., liquid, solid, or gas). The signal beams are then generated by the scattering of the input beams off the grating. The intensity of each phase conjugate signal beam is proportional to the square of the absorption coefficient of the analyte present at the point at which the two input beams are focused, and to the cube of the total intensity of the sum of the two input beams. The absorption coefficient of the analyte may be used to detect trace concentrations of particular substances in known fashion. Thus, by monitoring the intensity of a signal beam generated by the interference pattern setup when two input beams intersect within an analyte, ultratrace concentrations of substances of interest may be detected.

The use of two input beams in accordance with the first embodiment of the present invention allows a single lens to be used to focus and mix the input beams simultaneously. Thus, the beam spot of each of the input beams can be focused to less than 34 μm. Such small beam spots allow the present invention to directly focus the input beams within a standard component of an analysis system, such as a capillary tube of a high power/high performance capillary electrophoresis (HPCE), a column of a high performance liquid chromatography (HPLC) system, or to directly probe a small location inside a gas-phase atomizer such as flame, dc plasma, graphite furnace, inductively coupled plasma for diagnostic studies with high spatial resolution. Due to the fact that the first embodiment of the present invention uses only two input beams, the first embodiment of the present invention is far easier to align than prior art system which require three input beams.

Furthermore, since the present invention does not rely on fluorescent properties of the analyte, any substance that absorbs light at the wavelength of the laser used can be directly detected. Furthermore, even substances that do not absorb light at convenient wavelengths (such as amino acids, which absorb light only at ultraviolet wavelengths) can be indirectly detected. In accordance with an indirect method of detection, a liquid is selected for having desirable D4WM parameters including absorption coefficient, since the absorption coefficient is the parameter that is to be used to determine the presence of the liquid. The liquid then yields a positive baseline value. The sample to be detected is then injected into the system. The presence of the sample can then be detected to a very precise degree by the change in the measured absorption coefficient of the liquid. Therefore, in accordance with the present invention, trace concentrations of virtually any substance may be detected, without the use of a laser emitting light at a wavelength suited to that particular substance, since the liquid is the signal generating medium. Furthermore, in accordance with the present invention, an inexpensive diode laser may be used to generate the input laser beams, thus reducing both the cost and the size of the present invention. The use of low-power diode lasers, HeNe lasers or other compact inexpensive lasers is possible in accordance with the present invention because F-D4WM requires significantly less laser power and offers easier mixing and handling of less than perfect laser beams. For example, laser beams that are poorly columnated or which are highly divergent are refocused in accordance with the two-input-beam embodiment of the present invention.

In accordance with the second embodiment of the present invention, the input laser beams and the signal beam of a forward-scattering degenerate four-wave mixing method are carried by fiber optic cables. The fiber optic cables are secured to a substrate or mount such that they are immobilized with respect to the other components of the invention, such as the lens, apertures and a sample cell. Thus, the alignment of the system is preset and needs no further adjustment.

In accordance with a third embodiment of the present invention, a three input beam backward degenerate four-wave mixing method is used in which the two input laser beams are directed by fiber optic cables from the laser light source to opposite sides of a sample cell, and the third input beam is directed by a fiber optic cable from the laser light source to the sample cell through a beam splitter and a lens. The signal beam is also directed via fiber optic cable toward the detector. Each of the fiber optic cables is secured to a substrate such that the fiber optic cable is immobilized with respect to each other component of the system. The use of fiber optic cables to direct the laser beams of a three input beam system is of particular value due to the difficulties encountered in aligning such three beam systems.

The details of the preferred embodiment of the present invention are set forth in the accompanying drawings and the description below. Once the details of the invention are known, numerous additional innovations and changes will become obvious to one skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic diagram of a prior art three-input-beam backward-scattering degenerate four-wave mixing setup.

FIG. **2***a* is a schematic diagram of a two-input-beam forward-scattering degenerate four-wave mixing arrangement in accordance with the preferred embodiment of the present invention.

FIG. **2***b* is a schematic diagram of a two-input-beam forward-scattering degenerate four-wave mixing arrangement coupled to a high performance capillary electrophoresis system.

FIG. **2***c* is a schematic diagram of a two-input-beam forward-scattering degenerate four-wave mixing arrangement coupled to a high performance liquid chromatography system.

FIG. **2***d* is an illustration of an alternative embodiment of the arrangement shown in FIG. **2***a* in which the laser beams are each transmitted through a fiber optic cable.

FIG. **2***e* illustrates a three input beam backward degenerate four-wave mixing arrangement in which fiber optic cables are used to transmit the laser beams.

FIG. **3** graphically illustrates the relationship between the total power of the sum of the input beams and signal beam power, using actual data points observed from the embodiment of the present invention illustrated in FIG. **2***a*.

FIG. **4** shows the quadratic dependence of the beam signal on eosin B concentration in a particular application of the present invention.

FIG. **5***q* illustrates a first input beam and a second input beam, which constructively interfere to form a thermal

grating, which then scatters the corresponding input beams to generate F-D4WM signal beams.

FIG. 5*b* illustrates the relationship between the input beams and the signal beams when the first input beam has a greater intensity then the second input beam.

FIG. 5*c* illustrated the relationship between the input beams and the signal beams when the second input beam has a greater intensity then the first input beam.

FIG. 6 is a photograph of the four laser spots formed by the embodiment the present invention illustrated in FIG. 2*a*.

FIG. 7 illustrates the dependence of signal and signal-to-noise ratio on the chopper modulation frequency.

Figures are not to scale. Like reference numbers and designations in the various drawings refer to like elements.

DETAILED DESCRIPTION OF THE INVENTION

Throughout this description, the preferred embodiment and examples shown should be considered as exemplars, rather than as limitations on the present invention.

Physical Configuration of the Preferred Embodiment.

FIG. 2*a* shows a schematic diagram of a two-input-beam forward-scattering degenerate four-wave mixing F-D4WM arrangement in accordance with the preferred embodiment of the present invention. It should be understood that the particular arrangement of reflectors and beam splitters is provided only as an exemplar, and is not intended to limit the scope of the present invention.

The present invention includes an excitation light source **201**, which is preferably a compact diode laser, such as is commonly known. Alternatively, a helium-neon laser, a continuous-wave argon ion laser or a Nd:YAG laser may be used. A laser beam **203** output by the light source **201** is preferably reflected by a reflector **205** and split by a beam splitter **207** to form a first **209** and a second **211** input beam. The second input beam **211** is reflected by a second reflector **213** such that the first **209** and second **211** input beams are preferably generally parallel. In accordance with the illustrated embodiment of the present invention, the intensity I_1, of the first input beam **209** with respect to the intensity I_2 of the second input beam **211** arriving at the sample cell, I_1:I_2, is approximately 7:3 to generate the signal in one direction only. The preferred ratio is 1:1 to generate two signal beams.

In the preferred embodiment, a third reflector **215** and a fourth reflector **217** redirect the first input beam **209** and the second input beam **211**, respectively, toward a single 100-mm focusing lens **219**. The focusing lens **219** preferably focuses and-mixes both input beams **209**, **211**. A sample cell **221** is placed at the lens' focal point. The diameter of both the first input beam spot and the second input beam spot on the sample cell **221** is approximately 34 μm in the preferred embodiment. The first input beam **209** and the second input beam **211** intersect inside the sample cell **221** with an intersect angle of approximately 1.5° or less in the preferred embodiment of the present invention. The small input beam spots allow the present invention to interface directly with systems in which an analyte is available in a small volume, such as the capillary tube of a high power/high performance capillary electrophoresis system (HPCE), the column of a high performance liquid chromatography (HPLC) system, or to directly probe a small volume inside a gas-phase atomizer such as flame, dc plasma, graphite furnace, inductively coupled plasma, with high spatial resolution in diagnostic studies.

In order to optimize the signal strength of a phase-conjugate signal which is generated, the difference in path lengths (or distances traveled) for first input beam **209** and the second input beam **211** are preferably kept to less than the coherence length of the laser. A device for amplitude modulating the second input beam **211**, such as a mechanical light chopper **223** (for example, Model 03-OC4000, manufactured and distributed by Photon Technology International Inc., Princeton, N.J.), or any solid state electronic light intensity modulation device, is used. A phase-conjugate signal beam **225** generated in the sample cell **221** is directed to a detector **227**, such as a photomultiplier tube (e.g., Model R928, manufactured and distributed by Hamamatsu Corp., Middlesex, N.J.) after passing through a lens **229** preferably having a 250-mm focal length and preferably a filter **231** which in the preferred embodiment is a 514.5 nm laser-line filter when using an argon ion laser. A small aperture **233** is preferably disposed in front of the detector **227** to minimize background noise due to the scattering of the two input beams **209** and **211**.

The electrical output signal **235** of the detector **227** is then preferably coupled to a current-to-voltage converter **237**, the output of which is preferably monitored by a lock-in amplifier (otherwise known as phase sensitive amplifier) **238** (such as Model 5207, manufactured and distributed by Princeton Applied Research, Princeton, N.J.). The output from the detector **227** may also be coupled to other processor components **241**, such as a strip-chart recorder, personal computer including an analog to digital converter, or any other such processing device. Control of the present invention may be performed by the same computer used to control a HPCE, HPLC, or atomizer system with which the present invention is being used.

The sample cell **221** is preferably the capillary of an HPCE system, the column of an HPLC system, or a gas-phase atomizer system (e.g., flame, de plasma, ICP plasma, graphite furnace). However, a rectangular glass flow cell with approximately a 0.1-mm optical path length (such as a Type 48, manufactured and distributed by Starna Cells, Inc., Atascadero, Calif.) may be used to measure an analyte for other purposes. Naturally, the sample cell **221** may take any form which can hold a volume of analyte which is at least equal to the spot volume of the focused input beams **209**, **211**, and which allows the input beams **209**, **211** to enter and the signal beam **225** to exit without excessive attenuation. Furthermore, the analyte in the sample cell **221** may be any substance in any phase (i.e., liquid, solid, or gaseous), such as eosin B dissolved in ethanol and iodine in carbon tetrachloride. The present invention is capable of analyzing solids and gases, as well as liquids.

An analyte solution is preferably delivered to the sample cell **221** in accordance with the system with which the present invention is being used. For example, the analyte is delivered by electrophoresis in an HPCE system or a pump in a HPLC system. Alternatively, a pump, such as a peristaltic pump **239** may be used to deliver the analyte to the sample cell **221**.

After the two input beams **209**, **211** pass through the sample cell **221**, they are blocked by a beam trap **243**, and the signal beam **225** is easily separated and directed toward the detector **227**. An analyte solution with a relatively high concentration (e.g., 5×10^{-6}M eosin B) is preferably used as an "alignment solution" to optimize the optical alignment. In accordance with the preferred embodiment of the present invention, a micromolar-level solution can generate a strong signal that is visible to the naked eye, thus allowing simple alignment of the present invention. Signal optimization is performed simply by adjusting the mirrors and the lenses, and by carefully adjusting the position of the sample cell **221**

so that the sample cell 221 is at the focal point of the wave-mixing lens, while observing the strength of the visible signal spot on a card (or on a photodetector for trace-concentration analytes). Of course, any other means for determining the maximum strength of the signal beam 225 while adjusting the alignment of the system would be equally acceptable. For example, a self-adjusting system using feedback from the lock-in amplifier 238 might be used to determine the optimum alignment of the system. Once the optical alignment is optimized, the alignment and the signal remain very stable and different analyte solutions could be flowed through and analyzed without any further adjustments.

FIG. 2*b* illustrates the two-input-beam embodiment of the present invention coupled to the capillary tube of a HPCE system. The sample cell 221 is part of the capillary tube of the HPCE system. One end of the sample cell draws from a positive pool 249. The other end of the sample cell discharges into a negatively charged pool 251. A high voltage source is coupled to, and controlled by, the HPCE system controller 247.

FIG. 2*c* illustrates the two-input-beam embodiment of the present invention coupled to a column 257 of a HPLC system. The sample cell 221 is coupled at one end to the column 257, and at the other end to a waste pool 259. A pump is coupled to, and controlled by, a HPLC system controller 255.

In an alternative embodiment of the present invention illustrated in FIG. 2*d*, the laser beams 209, 211, 225 are each transmitted through a fiber optic cable 250. In such embodiments, transmission of laser light through air is preferably minimized. The fiber optic cable may be prealigned and bound to a substrate to prevent misalignment. In such an embodiment, the output of the laser source 201 is coupled directly to the fiber optic cable 250 in known fashion. The fiber optic cable is split in two sections 253, 255 in known fashion, thus dividing the beam into the first input beam 209 and the second input beam 211. The second section of fiber optic cable 255 is preferably coupled to an amplitude modulation device 223', such as a well known mechanical chopper, or any solid state electronic light intensity modulation device or an electro-optical modulator. Use of an electronic circuit for modulating the second input beam 211 allows the system to be produced in a compact package. The output of the first section of fiber optic cable 253 and the output from the modulation circuit 223' are preferably coupled to a lens 219. The lens causes the two input beams 209, 211 to be focused to a fine point within a sample cell 221. The input beams 209, 211 are preferably trapped at the opposite side of sample cell 221 by a beam trap 243. A signal beam 225 is generated within the sample cell 221and projects outward through an aperture 233, a lens 229, a line filter 231, and into a photomultiplier tube 227. The path from the sample cell 221 to the photomultiplier tube 227 may, in one embodiment of the present invention, be through a fiber optic cable 228. Alternatively, the path may be through air. In one alternative embodiment of the present invention, two signal beams 225, 303 (see FIG. 5*a*) may be coupled to the photomultiplier tube 227 through a summing lens 259 by fiber optic cable 261, or each signal beam 225 and 303 may be coupled to a separate photomultiplier or photodiode, via air or fiber optic cables and detected by summing or multiplication.

The use of fiber optic cable to minimize alignment difficulties has particular application to backward D4WM systems, such as the system illustrated in FIG. 1. A system such as that illustrated in FIG. 1 which utilizes fiber optic cables is illustrated in FIG. 2*e*, a fiber optic cable 130 is divided into three 137, 139, 141 such that the signal is split into three beams 107, 109, 117. One input beam 107 is preferably directed to the sample cell 113 at the appropriate angle by the fiber optic cable 139. The second input beam 117 is directed by the fiber optic cable 141 at a modulation device 118 and a beam splitter 123 which passes the entire input beam 117. The input beam 117 is focused by a lens 122. A signal beam 121 is generated within the sample cell 113 and transmitted along the path that the input beam 117 traverses from the beam splitter 123. Upon striking the beam splitter 123, the signal beam 121 is reflected toward an aperture 125. The signal beam 121 can be directed toward the photodetector via fiber optic cable or through air.

The input beam 109 is transmitted from the laser light source to the sample cell 113 by fiber optic cable 137 at the appropriate angle to counterpropagate the input beam 107 and to cause the desired signal beam 121 to be generated.

Theory Underlying the Present Invention

In an absorbing medium, the phase-conjugate signal beam 225 is generated mostly by thermally induced nonlinear effects. When two coherent beams 209, 211 intersect in the absorbing medium, thermally induced refractive index change is obtained following the absorption of input photons by the analyte species and the subsequent thermalization via rapid radiationless relaxation of optically excited analyte species. Hence, a thermally induced refractive index grating is formed, from which input beams 209, 211 are scattered to produce the phase-conjugate signal beams 225, 303, respectively. The resulting F-D4WM signal intensities, I_3 and I_4, can be expressed as:

$$I_3 = CI_1^2 I_2 (\lambda^2/\sin^2\theta)[dn/dT]^2 m^2 [\alpha/\kappa]^2$$

$$I_4 CI_1 I_2^2 (\lambda^2/\sin^2\theta)[dn/dT]^2 m^2 [\alpha/\kappa]^2$$

where C is a constant, dn/dT is the temperature coefficient of the refractive index, m is the fringe modulation level, α is the absorption coefficient of the nonlinear analyte medium, and k is the thermal conductivity.

Equations 1 and 2, illustrate several important characteristic properties of the signal beam 225, including quadratic dependence of the signal beam 225 on the intensity I_1 of the input beam 209, quadratic dependence of the signal beam 225 on the refractive-index change due to temperature change, and quadratic dependence of the signal beams 225 on the absorption coefficient. Since each of the other variables are known to a high degree of accuracy, the absorption coefficient can be determined to a high degree of accuracy. Determining the absorption coefficient of the solution within the sample cell allows a determination to be made as to the composition of the solution.

Equation 1 also indicates that the intensity I_3 of the signal beam 225 has a linear dependence on the intensity I_2 of the second input beam 211, a quadratic dependence on the intensity I_1 of the first input beam 209, and hence, a cubic dependence on the total intensity I_t of all input beams 209, 211. Similarly, equation 2 shows that the intensity I_4 of the signal beam 303 has a linear dependence on the intensity I_1 of the first input beam 209, a quadratic dependence on the intensity I_2 of the second input beam 211, and hence, a cubic dependence on the total laser intensity I_t of all input beams.

FIG. 3 graphically illustrates the relationship described in equation 1, using actual data points observed from the embodiment of the present invention illustrated in FIG. 2*a*. Using the embodiment of the present invention shown in FIG. 2*a*, the signal beam 225 is shown to have a linear dependence (slope=0.96) on the intensity I_2 of the input

beam 211, a quadratic dependence (slope=1.98) on the intensity I_1 of the input beam 209, and a cubic dependence (slope=3.03) on the total input laser intensity I_t.

Alternatively, the signal beam 303 has a linear dependence on the intensity I_1 of the input beam 209, a quadratic dependence on the intensity I_2 of the input beam 211, and a cubic dependence on the total input laser intensity I_t. This nonlinear dependence of signal on laser intensity is one of many important characteristics of the F-D4WM method that yield excellent detection sensitivity. For instance, an order of magnitude increase in total laser intensity would result in a 3 orders of magnitude increase for the F-D4WM signal. A good signal to noise ratio is obtained in the present invention despite this non-linear dependence upon the total intensity of the laser beam and the resulting amplification of the fluctuations of intensity of the laser beam, due to (a) the availability of enormous amounts of signal intensity and (b) the signal collection efficiency that is virtually 100% (i.e., signal is a laser beam). In addition, other types of noise, such as background scattering off of optics, increase only linearly with laser power while the signal increases as the cube of the laser power. Hence, the net gain in signal-to-noise ratio is better than that of conventional methods, making it possible for the nonlinear laser method of the present invention to yield excellent S/N and detection limits.

FIG. 4 shows the quadratic dependence of the beam signal 225 on eosin B concentration in a particular application of the present invention. The quadratic dependence of the beam signal 225 on the eosin B suggested by equations 1 and 2 is shown in FIG. 4. Eosin B is used as an example of an analyte only for convenience. Detection of eosin B is important, since eosin B is currently used in many applications in which detection of trace concentrations of eosin B is important, such as labeling proteins or other molecules and artificial food coloring. However, a major advantage of the present invention is the fact that a very wide range of analytes can be detected, including both fluorescing and non-fluorescing analytes. The only requirement to "direct" detection of an analyte by F-D4WM is the absorption coefficient of the analyte. Even in the case in which a particular sample has poor absorption characteristics (e.g., many amino acids absorb predominately at ultra-violet wavelengths), an "indirect" method of detection may be employed.

In accordance with an indirect method of detection, a liquid is selected for having desirable D4WM characteristics, such as absorption coefficient, since the absorption coefficient is one of the parameters that is to be used to determine the presence of the liquid. Other parameters include third order nonlinear susceptibility, $\chi^{(3)}$, the refractive index change based on temperature change, dn/dt, and the refractive index, n. The liquid then yields a positive baseline value for the absorption coefficient of the liquid within the sample cell. The sample to be detected is then injected into the system. The presence of the sample can then be detected to a very precise degree by the change in the measured absorption coefficient of the liquid within the sample cell.

The use of such an indirect method for detecting the presence, concentration, or mass of a chemical provide the following benefits: (1) the analysis is applicable to a larger number of substances; (2) a single fixed wavelength laser may be used for any analyte; (3) the wavelength of the laser may be selected to the user's convenience; (4) the maximum absorption wavelength of an analyte may be more than 100 nm away; (5) universal detection of many unlabeled analytes which would otherwise require labeling; (6) neither the solvents nor the analytes require derivitization; and (7) femtomole/attomole-level detection sensitivity is possible.

FIG. 5*a* illustrates a first input beam 209 and a second input beam 211, which constructively interfere to form a thermal grating, which then scatters the corresponding input beams 209, 211 to generate F-D4WM signal beams, 225, 303. Note that in the above description of the embodiment of the present invention, only one of the signal beams 225 was discussed, since only one such signal beam 225 is of practical interest. However in embodiments of the present invention in which fiber optic cables are used, both signal beams 225, 303 may be generated, summed and/or multiplied. Placing a screen behind the sample cell 221 allows four laser spots formed by the four beams 209, 211, 255, 303 to be observed. FIG. 6 is a photograph of the four laser spots formed by the illustrated embodiment the present invention. Two bright spots 401, 403 are due to the input beams 209, 211, and two smaller spots, 405, 407 are due to the signal beams 225, 303, respectively.

When both input beams 209, 211 have the same intensity, two signal beams 225, 303 with the same intensity are observed. Such is the case illustrated in FIG. 5*a*. K_1, K_2, K_3, and K_4 represent the beam vector of four beams, 209, 211, 225, 303, respectively. If the intensity of the first input beam 209 is much stronger than that of the second input beam 211, the signal beam 225 is much stronger than the signal beam 303, as shown in FIG. 5*b*. Similarly, if the intensity of the second input beam 211 is much stronger than that of the first input beam 209, then the signal beam 303 is much stronger than the signal beam 225, as shown in FIG. 5*c*.

Generation of signal beams 225, 303 in a F-D4WM setup can be described in terms of dynamic holography. Constructive interference between the first input beam 209 and the second input beam 211 creates a dynamic grating with a period of P, where $P=\lambda/[2\sin(\Theta/2)]$, λ is the laser wavelength, and θ is the angle between the two input beams. The signal beams, 225, 303 are then generated by the scattering of the input beams 209, 211, respectively, off the grating. As required in all nonlinear four-wave mixing processes, conservation of both energy and momentum must be satisfied in F-D4WM experiments for effective energy transfer. The energy conservation requires that

$$\omega_3=2\omega_1-\omega_2=2\omega-\omega=\omega \tag{3}$$

and

$$\omega_4=2\omega_2-\omega_1=2\omega-\omega=\omega \tag{4}$$

Since both incident beams 209, 211 have the same frequency, the signal beams 225, 303 also have the same frequency. Furthermore, momentum conservation requires that

$$K_3=2K_1-K_2 \tag{5}$$

and

$$K_4=2K_2-K_1 \tag{6}$$

Equations 5 and 6 indicate that the F-D4WM signal beam is most effectively generated in the K_3 and K_4 directions, as shown in FIG. 5*b* and 5*c*. Experimental results agree with Equations 5 and 6 as shown in FIG. 6.

Although only two input beams are used in the F-D4WM method of the present invention shown in FIG. 5*b* and 5*c*, they are still considered four-wave mixing methods, since the input beam 209 having the greater intensity provides two waves, the input beam 211 having the lesser intensity provides the third wave, and the signal beam 225 becomes the fourth wave, making the process a four-wave mixing

method. The fact that the input beam having the greater intensity provides two waves accounts for the factor of two in equations 5 and 6. In FIG. 5*b*, the more powerful input beam 209 serves as the pump beam that consists of two forward waves, and the less powerful input beam 211 serves as the probe beam that generates the signal beam 225. Similarly in FIG. 5*c*, the more powerful input beam 211 serves as the pump beam that consists of two forward waves and the less powerful input beam 209 serves as the probe beam that generates the signal beam 303. ΔK represents vector difference between (1) the sum of the probe beam vector and the signal beam vector, and (2) twice the pump beam vector ($\Delta K=K_2+K_3-2K_1$). ΔK is due to the angle between the pump and probe beams, the difference in the input wave arrival times or the difference in the input beam path length, and the ratio of the intensity of the pump beam to the intensity of the probe beam.

The signal beam 225 of the present invention can be enhanced by optimizing several parameters, including total laser intensity I_t. The efficiency of a F-D4WM grating, formed by the two input beams 209, 211 inside a nonlinear medium, such as a liquid analyte, is strongly affected by (1) the angle e between the two input beams, (2) the difference in input wave arrival times or the difference in input beam path length, and (3) the beam intensity ratio of the two input beams. Therefore, the angle Θ between the two input beams is preferably kept as small as possible in order to obtain optimum phase matching especially when using a thin sample cell 221. In the preferred embodiment of the present invention, the angle between the input beams 209, 211, is less than 1–5 degree. In addition, the smaller the angle θ between the two input beams 209, 211, the wider and sharper the F-D4WM grating period will be. A grating with a larger period is less vulnerable to grating washout due to thermal motion and external disturbance such as flow turbulence. Therefore, in accordance with the preferred embodiment of the present invention, better grating efficiency and higher signal strength can be expected with smaller angles Θ between the two input beams 209, 211. Furthermore, a smaller angle θ also increases the beam interaction volume and, hence, strengthens the F-D4WM signal. Therefore, in accordance with the preferred embodiment of the present invention, reducing the angle θ significantly increases the strength of the signal beam 225. When applying the present invention to detect concentrations of trace analytes, an angle as wide as 1.5° typical yields excellent detection limits. Therefore, optical alignment requirements are not severely restricted. Small-angle alignments are especially easy to perform in this two-input beam F-D4WM setup, since a single focusing lens is preferably used to focus all input beams 209, 211 instead of two or three lenses usually required in a three-input-beam D4WM method.

Another important parameter that could affect the grating sharpness is the difference between the path lengths or arrival times of the two input beams 209, 211. Optimum constructive interference between the two input coherent beams 209, 211 can be obtained in the preferred embodiment when the phase matching of the waves is at a maximum. Hence, it is preferable to keep the difference between the path lengths shorter than the laser coherence lengths ($L=c/n\Delta v$) in order to form sharp F-D4WM gratings. Many popular lasers offer sufficient room for convenient optical alignments and may be used in accordance with the present invention. For example, a simple argon ion laser has a relatively long coherence length (e.g., 5 cm) and more sophisticated narrow-bandwidth lasers, such as dye lasers, have much longer coherence length or coherence time.

The F-D4WM signal-to-noise ratio of the present invention can be optimized significantly in accordance with the preferred embodiment of the present invention by carefully adjusting the intensity distribution ratio for the two input beams 209, 211. In a F-D4WM setup where the signal beam 225 is monitored, as shown in FIG. 2*a*, a stronger signal beam 225 is obtained when the input beam 209 is stronger than the input beam 211, since the signal beam 225 is generated by first input beam 209 scattering off the thermal grating formed by the first input beam 209 and the second input beam 211. However, using a stronger first input beam 209 also increases the background noise due to the scattering of the beam 209 itself. Therefore, it is preferable to use an optimum intensity ratio $I_1:I_2$ of 7:3 for the input beam 209 in order to obtain both maximum signal strength and minimum background noise, where I_1 is the intensity of the input beam 209, and I_2 is the intensity of the input beam 211.

In accordance with the preferred embodiment of the present invention, to improve signal-to-noise ratio, background noise is suppressed by using an amplitude-modulated detection scheme. If the signal beam 225 is monitored as shown in FIG. 2*a*, most of the background noise comes from the scattering of the input beam 209. Therefore in the preferred embodiment of the present invention, the input beam 211 is modulated instead of the input beam 209 in order to obtain maximum noise suppression. Alternatively, if the signal beam 303 is monitored, the input beam 209 is modulated instead of the input beam 211, in order to obtain maximum suppression of noise originating most from the input beam 211.

FIG. 7 illustrates the dependence of signal and signal-to-noise ratio on the chopper modulation frequency for one embodiment of the present invention analyzing one particular analyte. By amplitude modulating the input signal 211 at the correct frequency, sufficient time is allowed for the grating to form completely, and all other amplitude modulation frequencies, including amplitude modulation frequencies at the chopper frequency which are out of phase with the chopper, can be filtered out by the lock-in amplifier 238. A signal line 240 couples the amplitude modulation device 223' to the lock-in amplifier 238. Frequency and phase information are passed over the signal line 240. The intensity of the signal beam 225 remains strong at low modulation frequencies, since sufficient time is allowed between on and off cycles for the F-D4WM grating to form completely. The intensity of the signal beam 225 decreases slowly as the modulation frequency is increased, since less time is available to form a complete and sharp grating. On the other hand, the background noise is suppressed to a greater degree by the lock-in amplifier 238 at higher modulation frequencies. Although the signal is weaker at higher modulation frequencies, the signal-to-noise ratio remains reasonably high and stable at higher frequencies. Hence, the present invention offers a wide usable modulation frequency range. The particular range will vary dependent upon the analyte. Alternatively, the amplitude of both the signal beams 225, 303 could be monitored and common mode noise subtracted by known techniques.

Although there are similar features between degenerate four-wave mixing methods and holography (or holography-like spectroscopic methods relying on two-color pumped-probed thermal gratings), the two processes are not identical. While holography is a sequential process where recording and reconstruction (writing and reading) steps are separate (or time delayed), these steps occur simultaneously in a degenerate four-wave mixing experiment. Therefore, while only two photons need to exist simultaneously in hologra-

phy, four photons must exist simultaneously in four-wave mixing methods. Another distinctive difference between holography and four-wave mixing is the different types of diffraction gratings generated. While only spatial diffraction gratings are involved in holography, both spatial diffraction gratings and temporal diffraction gratings could be used in four-wave mixing. The B-D4WM complex signal vector amplitude can be described as $P^3 \propto (Ae_be_p^*.e_f+Be_fe_p^*.e_b+Ce_p^*e_fe_b)E_fE_bE_p^*$, where A, B, and C are constants depending on the nonlinear susceptibility of the analyte and e_p e_b, e_p are polarization-state vectors of the forward, backward, and probe beams, respectively. In the equation above, the first and second terms describe contributions from the volume gratings formed by forward-probe and backward-probe beam pairs, respectively. These two terms can be used also to describe processes in holography. However, the third term describes contribution from the temporal grating (i.e., coherent gratings), and this grating is characteristic of B-D4WM. Another important and useful characteristic property of both forward- and backward-scattering four-wave mixing methods is the phase-conjugate property of the signal beam. The phase-conjugate property in the illustrated forward-scattering D4WM signal beam is verified by placing a thin wire in the probe beam path and observing its image on a white screen that is positioned in the signal beam path. The image of the object (i.e., a wire or anything small that can be placed inside the probe beam path) is "carried" and projected very clearly on the screen by the phase-conjugate signal beam when the distance between the image screen and the nonlinear medium (i.e., analyte cell) is the same as the distance between the object and the analyte cell. The phase-conjugate property of the signal beam generated by the analyte (i.e., the nonlinear medium) in a D4WM method has many potential applications including autocorrection of beam distortion or optical aberration.

The use of a single short-focal length lens for all the input beams not only simplifies focusing and mixing simultaneously, it also maximizes photon density available at the sample cell significantly, and hence, allows the use of low-power (milliwatts or less) lasers in a nonlinear spectroscopic method. In the illustrated two-beam F-D4WM setup, the signal beam **225** is still strong even when laser power as low as 5 mW is used. Tight focusing and compact wave mixing available with the inventive two-beam F-D4WM configuration reduces the laser power requirements sufficiently to allow lowcost lasers, such as He-Ne lasers or diode lasers, to be used as excitation light sources in the present invention. Furthermore, because the beam spots of the input beams **209**, **211** are very compact, the present invention may be directly interfaced to known HPCE, HPLC, and atomizer systems such that the laser mixing can be accomplished directly in the capillary tube or column (i.e., the capillary tube or column is the sample cell).

The present invention provides an ultrasensitive detection method for analytes in any physical state (e.g., liquid, gas, or solid) using laser powers at the milliwatt range. Since only two input laser beams are involved in this four-wave mixing process, the optical alignment is much easier than other four-wave mixing methods, no critical optical alignment constraints exist, and the beam spots are easily focused to a sufficiently small volume to allow detection directly with an HPCE, HPLC, or gas-phase atomizer setup. Improved sub-attomole detection sensitivity, picoliter probe volume, simple one-laser one-wavelength setup, and convenient analyte introduction make the present invention applicable to may problems, including relatively inexpensive, simple, ultrasensitive detection for use in HPCE, HPLC, or gas-phase atomizer systems. The laser power requirement for producing a strong F-D4WM signal is unusually low (milliwatt or less) compared to conventional backward-D4WM experiments. Furthermore, in accordance with the present invention, simple diode lasers may be used in place of larger, more expensive lasers, such as argon-ion lasers.

In one embodiment of the present invention, a preliminary F-D4WM detection limit of 7.1×10^{-9}M (S/N=2) for eosin B is determined using a 0.1-mm-thick sample cell and a total argon ion laser power of 0.5 W. This corresponds to a mass detection limit of 7×10^{-19} mol of eosin B within a detection probe volume of 98 pL. Using the same F-D4WM setup, a detection limit of 4.6×10^{-7}M or 45×10^{-18} mol for iodine in CCl_4 is also determined.

Table 1 compares the results of the present invention to four-wave mixing laser spectroscope methods using different configurations. The present invention requires a much simpler optical setup and a much lower laser power and yet yields comparable detection limits as compared to backward-scattering D4WM methods. When compared to a B-D4WM method using a pulsed excimer-pumped dye laser, the present invention yields an iodine mass detection limit that is 351 times better, and does not require a more expensive dye laser. When compared to a laser-induced thermal diffraction method using both an argon ion laser and a He-Ne laser, the present invention yields an iodine concentration detection limit that is 1.8 times better (and an iodine mass detection limit that is 3067 times better), and yet it employs a simpler optical setup with only a single laser instead of two different lasers. Thus, the present invention is easier to use and less expensive.

Conclusion

A two-input beam F-D4WM optical setup offers several advantages as a novel nonlinear laser spectroscopic method. Compared to backward-scattering D4WM and other four-wave mixing methods, where three input beams are used, the optical alignment of a two-beam F-D4WM method is significantly easier. For example, in a "boxcar" F-D4WM configuration, where two pump beams and one probe beam are required, the optical alignment is not as simple. Since only two input laser beams **209**, **211** are used and the signal beam **225** is visible to the naked eye, the optical alignment of this nonlinear F-D4WM laser system in accordance with the present inventive method is relatively simple, even when compared to many conventional one-beam or two-beam laser methods. In addition, a single lens may be used to focus all input beams. Furthermore, a single lens may be used to mix all input beams. Still further, in accordance with one embodiment of the present invention, fiber optic cables are used to further simplify alignment. Use of fiber optic cables to simplify the alignment of a D4WM detection system may be used with both backward and forward-scattering D4WM systems. Since the alignment of the present invention is simplified, the setup has a stable optical alignment. Therefore, there is little or no need to realign the system at regular relatively short intervals. Also, the two-wave input beam embodiment of the present invention has high wave-mixing efficiency inside a small volume with single short-focal-length focusing.

In addition, the efficiency of three beam wave-mixing methods strongly depends on the angle of the probe beam relative to the thermal grating for Bragg scattering condition, although the effect of the angle between the two pump beams is negligible.

Furthermore, these three input beams must be perfectly overlapped in order to obtain maximum wave-mixing effi-

ciency, and hence, optical alignment is more complicated for a "boxcar" F-D4WM method as compared to that for a two-beam F-D4WM method.

In addition to simpler input beam alignments, it is also easier to mix waves with high efficiency in a two-beam F-D4WM method, since only a single lens is needed to focus and mix all the input beams as shown in FIG. 2*a* (instead of two or three lenses needed in a B-D4WM method). Hence, it is relatively easy to use microsample cells or many popular small-diameter capillary flow cells for a two-beam F-D4WM detector. In the illustrated two-beam F-D4WM setup, the diameter of the focused beam spots at the sample cell is 34 μm or less, and hence, the laser probe volume inside the sample cell is 98 pL or less. Therefore, this F-D4WM detection method has many potential applications as an ultrasensitive detector for both fluorescing and nonfluorescing analytes for a capillary chromatography system or a capillary electrophoresis system. As indicated in equation 2, the F-D4WM signal has a cubic dependence on total input laser intensity, and hence, the signal could be enhanced significantly by increasing the input intensity. Therefore, the present invention has low laser power requirements due to the high wave mixing efficiency and high photon density available by tight focusing using a short focal-length lens. Since the laser power requirements are low, and the input beams are refocused, the present invention may take advantage of inexpensive light sources, such as diode lasers. Also, the present invention is compact and portable.

The D4WM detection method of the present invention is suitable for ultrasensitive analytical measurements, since they can yield sub-attomole mass detection sensitivity and they are capable of detecting both fluorescing and nonfluorescing analytes.

A number of embodiments of the present invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. For example, the present invention may be used as a component part of any chemical analysis instrument system in which it is desired to detect the atomic or molecular absorption of a substance. For example the present invention may be used with any chemical separation instrument including liquid and gas chromatography HPCE and any optical spectrometry method in which the signal is based on absorption of light by the analyte. Furthermore, the present invention may employ any coherent light source capable of 1 milliwatt or less. Still further, the illustrated embodiments of the present invention are described as having an amplitude modulation device. In an alternative embodiment, the present invention may use a two channel detect and subtract method by which intensity variations or noise that are common each input beam are detected and subtracted from the signal beam. However, embodiments which have no such amplitude modulation or other signal to noise enhancement device are within the scope of the present invention. Furthermore, the present invention may be used with any combination of beam splitters, reflectors, lens, apertures, and other standard components of a laser or optical system, in order to accomplish the illustrated two input beam F-D4WM, or when used with fiber optic cables to direct beams of laser light, in order to accomplish the illustrated three beam B-D4WM. Accordingly, it is to be understood that the invention is not to be limited by the specific illustrated embodiment, but only by the scope of the appended claims.

I claim:

1. An apparatus for performing two-input-beam forward-scattering degenerate four-wave mixing for use in detecting absorption of light by an analyte, including:

(a) a sample cell for containing an analyte; and

(b) a coherent light source having a coherence length, the coherent light source for generating a first and a second input coherent beam, each having respective incident paths and a path length from the coherent light source to the sample cell, the first input coherent beam path length minus the second input coherent beam path length being less than or equal to the coherence length of the coherent light source, the coherent light source being configured such that the first and second input coherent beams intersect within the analyte at an angle θ of less than approximately 5 degrees so as to generate a first and a second signal beam each having a collimated coherent beam generated by interaction of at least one of the first and second input coherent beams with a thermally induced refractive index grating formed by constructive interference of the first and second input coherent beams, the first and second signal beams having respective signal paths, the signal path of the first signal beam being at an angle from the incident path of the second input coherent beam approximately equal to θ, and the signal path of the second signal beam being at an angle from the incident path of the first input coherent beam approximately equal to σ; and

(c) a detector disposed within the signal path of at least one signal beam, for receiving the coherent beam of such signal beam and determining from such signal beam absorption of light by the analyte in the sample cell..

2. The apparatus of claim 1, further including at least one lens through which each input coherent beam passes for focusing each input coherent beam to a single point within the sample cell.

3. The apparatus of claim 1, further including at least a first lens through which the first input coherent beam passes and a second lens through which the second input coherent beam passes, each lens having a common focal point, the common focal point being within the sample cell.

4. The apparatus of claim 1, wherein a ratio of the intensity of the first input coherent beam to the intensity of the second input coherent beam is approximately 7:3.

5. The apparatus of claim 1, further including an amplitude modulation device, disposed within the path of the second input coherent beam between the coherent light source and the sample cell for amplitude modulating the intensity of the second input coherent beam.

6. The apparatus of claim 5, wherein the amplitude modulation device is a solid state electronic light intensity modulation device.

7. The apparatus of claim 5, wherein the amplitude modulation device is a mechanical chopper.

8. The apparatus of claim 1, further including a first beam splitter for dividing the output of the coherent light source into the first input coherent beam and the second input coherent beam.

9. The apparatus of claim 8, further including:

(a) a first reflector disposed between the coherent light source and the first beam splitter;

(b) a second reflector for redirecting the first input coherent beam toward a lens;

(c) a third reflector for redirecting the second input coherent beam, such that the second input coherent beam propagates along a path that is approximately parallel to the path of the first input coherent beam between the beam splitter and the second reflector;

(d) a fourth reflector for redirecting the second input coherent beam toward the lens.

10. The apparatus of claim 1, further including:

(a) at least one lens; and

(b) a fiber optic cable;

wherein the output of the coherent light source is coupled to the fiber optic cable, the fiber optic cable being divided into two sections, the first section of cable for transmitting the first input coherent beam to a lens, the second section of cable for transmitting the second input coherent beam to a lens.

11. The apparatus of claim 10, wherein the lens to which the first input coherent beam is transmitted is at an end of the first section of fiber optic cable, and the lens to which the second input coherent beam is transmitted is at an end of the second section of fiber optic cable.

12. The apparatus of claim 11, further including:

(a) an amplitude modulation device for amplitude modulating the intensity of the second input coherent beam; and

(b) a fiber optic cable, coupled to the output of the coherent light source, and divided into two sections, the first section of cable for transmitting the first input coherent beam to the lens, the second section of cable for transmitting the second input coherent beam to the amplitude modulation device.

13. The apparatus of claim 11, further including a second lens, wherein the first signal beam and the second signal beam are summed within the second lens.

14. The apparatus of claim 11, further including:

(a) an aperture disposed within the signal path of the first signal beam for reducing background noise;

(b) a lens disposed within the signal path of the first signal beam for focusing the first signal beam;

(c) a filter disposed within the signal path of the first signal beam for attenuating light having a wavelength other the wavelength of the light emitted by the coherent light source; and

(d) a detector disposed within the signal path of the first signal beam for receiving the coherent beam of such first signal beam and converting the coherent beam of such first signal beam into an electrical signal.

15. The apparatus of claim 14, wherein the detector is a photomultiplier tube.

16. The apparatus of claim 14, further including an amplifier for amplifying the electrical output of the detector.

17. The apparatus of claim 16, wherein the gain of the amplifier is frequency dependent, and the gain is greatest at the amplitude modulation frequency.

18. The apparatus of claim 1, wherein the sample cell is a capillary tube of a high power/high performance capillary electrophoresis system.

19. The apparatus of claim 1, wherein the sample cell is a column of a high performance liquid chromatography system.

20. The apparatus of claim 1, further including a detection device disposed in the signal path of the first signal beam for detecting the intensity of the first signal beam.

21. The apparatus of claim 20, wherein the detection device includes a photomultiplier tube.

22. The apparatus of claim 21, further including a line filter for attenuating light at wavelengths other than approximately a wavelength of the coherent light source, disposed between the sample cell and the photomultiplier tube.

23. The apparatus of claim 22, further including a beam trap disposed with respect to the sample cell such that the first and second input coherent beams are trapped after passing through the sample cell.

24. The apparatus of claim 20, further including an aperture disposed between the sample cell and the detection device for reducing background noise.

25. The apparatus of claim 20, further including an amplifier coupled to the detection device for amplifying an electrical output of the detection device.

26. The apparatus of claim 25, further including an amplitude modulation device disposed in the incident path of the second input coherent beam between the coherent light source and the sample cell, wherein the gain of the amplifier is frequency dependent and is greatest at the modulation frequency of the amplitude modulation device.

27. The apparatus of claim 1, wherein the sample cell is coupled to a source of the analyte and a sink for the analyte, such that the analyte flows through the sample cell.

28. The apparatus of claim 27, further including a pump coupled to the sample cell for causing the analyte to flow through the sample cell.

29. The apparatus of claim 1, wherein the coherent light source is a diode laser.

30. The apparatus of claim 1, wherein beam spots for the first and the second input coherent beams within the analyte are less than 34 μm.

31. The apparatus of claim 1, wherein the intersecting angle of the first and second input coherent beams is in the range of 0.5 to 1 degree.

32. An apparatus for performing three-input-beam backward degenerate four-wave mixing for use in detecting atomic absorption of light by an analyte, including:

(a) a coherent light source;

(b) a beam splitter;

(c) a lens;

(d) a sample cell for containing an analyte; and

(e) a fiber optic cable divided into three sections for dividing an output of the coherent light source into three input beams, the first section of fiber optic cable for directing the first input beam to strike a first side of the sample cell, the second section of fiber optic cable for directing the second input beam to strike a second side of the sample, cell opposite the first side, and the third section of fiber optic cable for directing the third input beam to pass through the beam splitter and the lens, and to intersect the first and second input beams at a point within the sample cell;

whereby a phase conjugate signal beam is generated within the analyte in response to constructive interference between the first and second input beams, and the signal beam reflects off the beam splitter.

33. The apparatus of claim 32, further including a detector for detecting the intensity of the signal beam, disposed to receive the signal beam reflected off the beam splitter.

34. The apparatus of claim 33, wherein the detector includes a photomultiplier tube.

35. The apparatus of claim 34, further including an amplifier coupled to the detector for amplifying the output of the detector.

36. The apparatus of claim 35, further including an amplitude modulation device disposed within the path of the third input signal.

37. The apparatus of claim 36, wherein the gain of the amplifier is frequency dependent, and the greatest gain is provided at frequencies near the modulation frequency of the modulation device.

38. The apparatus of claim 36, wherein the amplifier is coupled to a processor for digitizing, recording, and processing the output of the amplifier.

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